PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

RIZZO, Thomas M.
E.I. du Pont de Nemours and Company
Legal Patent Records Center
1007 Market Street
Wilmington, DE 19898
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 11 January 2001 (11.01.01)	
Applicant's or agent's file reference BB1355 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US00/12061	**International filing date** (day/month/year) 03 May 2000 (03.05.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
GEIGER, Kathleen, W. E.I. du Pont de Nemours and Company Legal Patent Records Center 1007 Market Street Wilmington, DE 19898 United States of America	Telephone No. 302 992 3749 Facsimile No. 302 773 0164 Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
RIZZO, Thomas M. E.I. du Pont de Nemours and Company Legal Patent Records Center 1007 Market Street Wilmington, DE 19898 United States of America	Telephone No. 302 992 3749 Facsimile No. 302 773 0164 Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☒ the International Searching Authority
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Lazar Joseph Panakal Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year) 11 January 2001 (11.01.01)	
International application No. PCT/US00/12061	**Applicant's or agent's file reference** BB1355 PCT
International filing date (day/month/year) 03 May 2000 (03.05.00)	**Priority date** (day/month/year) 07 May 1999 (07.05.99)
Applicant OROZCO, Emil, M., Jr. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 November 2000 (20.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

RIZZO, Thomas M.
E.I. du Pont de Nemours and Company
Legal Patent Records Center
1007 Market Street
Wilmington, DE 19898
ETATS-UNIS D'AMERIQUE

RECEIVED
AUG 20 2001
PATENT RECORDS CENTER

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	07.08.2001
Applicant's or agent's file reference BB1355 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/12061	03/05/2000	07/05/1999

Applicant
E.I. DU PONT DE NEMOURS AND COMPANY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hingel, W Tel.+49 89 2399-8717

REY NOTED

Form PCT/IPEA/416 (July 1992)

07 NO 2001

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BB1355 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/12061	International filing date *(day/month/year)* 03/05/2000	Priority date *(day/month/year)* 07/05/1999

International Patent Classification (IPC) or national classification and IPC
C12N15/29

Applicant
E.I. DU PONT DE NEMOURS AND COMPANY et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☒ Certain observations on the international application

Date of submission of the demand 20/11/2000	Date of completion of this report 07.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Rojo Romeo, E Telephone No. +49 89 2399 7321

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/12061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

   Description, pages:

   1-37 as originally filed

   Claims, No.:

   1-24 as originally filed

   Drawings, sheets:

   1/4-4/4 as originally filed

   Sequence listing part of the description, pages:

   1-45, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-24 (partially).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-24 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-24 (partially).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24 (partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24 (partially)
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-24 (partially)

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of this report

Since the applicant did not file an answer to the Written Opinion, the present International Preliminary Examination Report is based on said opinion.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability.

Since the Applicant failed to pay additional search fees, claims 1-24 were only searched as far as they concerned SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 ,12, 13, 14, 47 and 48.

Examination is thus carried out on claims 1-24 (partially as far as they concern these sequences.

Re Item IV

Lack of unity of invention

According to Rule 13 PCT an application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept, i.e. having at least one common technical feature defining a contribution over the known prior art.

The International Preliminary Examination Authority agrees with the objection raised for lack of unity by the International Search Authority. The ISA found that the present application concerns 4 groups of inventions:

invention 1 (claims 1-24, partially)

An isolated polynucleotide derived from maize selected from the group of: a nucleotide sequence encoding a polypeptide of at least 30 amino acids that is at least 85% identical to the sequence set forth in SEQ ID NO: 6, of at least 50 amino acids being at least 85% identical to SEQ ID NO: 12, of at least 50 amino acids being at least 90% identical to SEQ ID NO: 8, of at least 1000 amino acids being at least 90%identical to SEQ ID NO: 2, of at least 150 amino acids being at least 95% identical to SEQ ID NO: 4, of at least 350 amino acids being at least 95% identical to SEQ ID O: 10, of at least 200 amino acids being at least 80% identical to SEQ ID NO: 14, or of at least 250 amino acids being at least 95% identical to SEQ ID NO: 48, or a polynucleotide sequence complementary thereto.

Said polynucleotide sequences comprising the sequences selected from SEQ ID NO: 1,

3, 5, 7, 9, 11, 13, 47, being DNA or RNA.
Chimeric genes, transformed host cells, proteins derived therefrom.
Use of said sequences in a method for modulating root development in plants, and for diagnostic screening purposes. Products derived from said diagnostic screening studies.

invention 2 (claims 1-24, partially)
idem for SEQ ID NO: 15-20, derived from rice.

invention 3 (claims 1-24, partially)
idem for SEQ ID NO: 21-34, derived from soybean.

invention 4 (claims 1-24, partially)
idem for SEQ ID NO: 35-42, 45, 46, derived from wheat.

Plant auxin transport proteins have been described and cloned in the prior art (see e.g. D1, D2, D5, D6), and used in plant genetic engineering.

In the light of the prior art, the problem underlying the present application is the provision of alternative plant auxin transport protein encoding sequences.
The solution to this problem as provided herein are the following:
1. solution: an isolated polynucleotide from maize selected from the group as defined and listed, or a polynucleotide sequence complementary thereto.
Chimeric genes, transformed host cells, proteins derived therefrom.
Use of said sequences in a method for modulating root development in plants, and for diagnostic screening purposes. Products derived from said diagnostic screening studies.
2. solution: idem for SEQ ID NO: 15-20, derived from rice.
3. solution: idem for SEQ ID NO: 21-34, derived form soybean.
4. solution: idem for SEQ ID NO: 35-42, 45, 46, derived from wheat.

Due to the prior art disclosing auxin transport proteins from plant sources, due to the essential difference of the primary structure of the claimed nucleic acid molecules and proteins of the present application, and due to the fact that no other technical features could be distinguished, which in the light of the prior art could be regarded as special technical features, the ISA was of the opinion that there was no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule

13.1 PCT. Consequently, there is lack of unity.

Since the Applicant failed to pay additional search fees, only the first group of inventions is considered for the examination.

The Applicant's attention is drawn to the fact that the first group of invention lacks unity and will be split, at the regional phase, into as may independent inventions as there are sequences claimed. Indeed, the common inventive concept linking the claims as far as they concern invention 1 is that the claimed nucleotide and polypeptide molecules are of maize origin. Nucleic acids and proteins of maize origin are known from prior art (e.g. D3, D4). Thus, this common inventive concept does not exist.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents cited in the International Search Report:

D1: DATABASE EBI [Online] AC O81215, 1 November 1998 (1998-11-01) LUSCHNIG ET AL.: 'Rice EIR1 homologue REH1' XP002146833

D2: LUSCHNIG C ET AL: 'EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana' GENES AND DEVELOPMENT,US,COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, vol. 12, no. 14, 15 July 1998 (1998-07-15), pages 2175-2187, XP002116368 ISSN: 0890-9369

D3: SCHWOB E ET AL: 'MOLECULAR ANALYSIS OF THREE MAIZE 22 KDA AUXIN-BINDING PROTEIN GENES - TRANSIENT PROMOTER EXPRESSION AND REGULATORY REGIONS' PLANT JOURNAL,GB,BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, vol. 4, no. 3, 1993, pages 423-432, XP002024567 ISSN: 0960-7412

D4: ZETTL R ET AL: '5' AZIDO-3 6-TRITIATED-1-NAPHTHYLPHTHALAMIC ACID A PHOTOACTIVATABLE PROBE FOR NAPHTHYLPHTHALAMIC ACID RECEPTOR PROTEINS FROM HIGHER PLANTS IDENTIFICATION OF A 23-KDA PROTEIN FROM MAIZE COLEOPTILE PLASMA MEMBRANES' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 2, 1992, pages 480-484, XP002146831 1992 ISSN: 0027-8424

D5: GÄLWEILER L. ET AL.: 'Regulation of polar auxin transport by AtPIN1 in

Arabidopsis vascular tissue' SCIENCE, vol. 282, December 1998 (1998-12), pages 2226-2230, XP002146832

D6: EP-A-0 814 161 (MAX PLANCK GESELLSCHAFT) 29 December 1997 (1997-12-29) cited in the application

1. Novelty (Art. 33(2) PCT)

None of the documents cited in the International Search Report discloses the claimed subject-matter. The current set of claims is thus considered novel over these documents, given that the clarity problems set below are relieved.

2. Inventive step (Art. 33(3) PCT)

2.1 As mentioned above, auxin transport proteins are known from prior art. The problem underlying the current application is the provision of alternative auxin transport proteins from maize. The solution provided by the current application is the provision of the DNA molecules of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, and 47. Although the application discloses the nucleotide sequence of these open reading frames (ORF), these sequences appear to be the "best guess" ORFs identifiable as putative auxin transport proteins. The Applicant fails, however, to provide tangible evidence of the expression of these proteins, of their identity as auxin transport proteins and of their role (the auxin transport proteins were shown to have varied phenotypes if disrupted). Indeed, these sequences appear to be the result of mere computer work (see examples) by sequence comparison for the identification of the homologs of the already known auxin transport proteins: for the person skilled in the art, the review of a gene database or an EST database as described in the present application (examples) belongs to standard techniques and does not involve any inventive activity. Hence, in the absence of experimental data, the DNA molecules and proteins putatively encoded claimed constitute "compounds" with no known technically useful property. In this case, any prior art compound identifiable as an auxin transport protein (see D1-D6), regardless of its technical properties, is equally suitable as the starting point for making structural modifications and may be considered to represent the closest prior art. Without the concomitant need to provide any particular technical effect, for the skilled person any putative auxin transport protein (see e.g. D4) may provide an equally obvious solution.

Consequently, the solution provided by the current application (SEQ ID NO: 1, 3, 5,

7, 9, 11, 13, and 47 and corresponding amino acid sequences 2, 4, 6, 8, 10, 12, 14, and 48) cannot be considered inventive.

Embodiments of these sequences and methods claimed are considered to be obvious to the skilled person and thus, not inventive.

The Applicant's attention is drawn to the fact that D4 also describes a maize protein which may be involved in auxin transport and thus gives a clear hint for auxin transport proteins to be found in this plant. Thus, also for this reason, the present application cannot be considered inventive over prior art since the skilled person would be prompted to look for auxin transport proteins in maize.

Consequently, claims 1-24 lack inventive step.

3. Industrial applicability (Art. 33(4) PCT)
The Applicant's attention is drawn to the fact that the claimed DNA and polypeptide molecules claimed lack technical effects. The putative functions of these different molecules were assigned by computer sequence analysis without providing actual proof of the function of any of these molecules. For the EPO, compounds without function are not considered to have an industrial applicability. Thus, the present set of claims has no industrial applicability.

<u>Re Item VIII</u>

Certain observations on the international application

1. Clarity (Art. 6 PCT)

1.1 Claim 1 is directed to "an" isolated polynucleotide encoding "a" polypeptide having homology to the claimed SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 and 48 without any technical effect since no function is indicated

1.2 Claim 11 is directed to a method of selecting an isolated polypeptide that affects the level of expression of "any" polypeptide. It is also unclear whether this should be a direct effect (peptide encoded by the 30 nucleotides?) or an indirect effect of said polypeptide on any other polypeptide.

The same objection applies to claims 14 and 15, since the polypeptide encoded by

the nucleic acid to clone has no particular function.

1.3 Concerning claim 24, the lack of reference to a particular sequence leads to a method for modulating root development in a plant using any auxin transport protein known, not only those claimed in the present specification. Consequently, an objection for lack of novelty may also arise.

2. Support by specification (Art. 6 PCT), in combination with Art. 5 PCT (complete and enabling disclosure)

2.1 Art. 6 PCT requires the claims to be fully supported by the description, i.e. the claims must be formally and technically supported by the description. The IPEA considers that the present description actually only discloses polynucleotides and bacterial host cells transformed with this polynucleotide (library clones). The description refers to the predicted proteins based only on the possible ORF of said polynucleotide. However, there is no real characterization of any protein (N-ter or C-ter sequence, complete amino acid sequence, etc), let alone of an "isolated polypeptide". Thus, the IPEA considers that such a subject-matter has no technical support. Furthermore, the fact that these putative polypeptides have a certain homology to other known proteins is far away from an actual characterization of any real "biological activity". Thus the IPEA considers that claim10, and dependent claim18 which refer to the putative amino acid sequence of proteins which have not been actually disclosed or well characterized and which have not been shown to have the claimed activity, are not supported by the description (Art. 6 PCT).

2.2 The present application is silent as far as the method claimed in claim 11 is concerned. Indeed, no basis is given for the influence of a polynucleotide of at least 30 nucleotides (derived from the polynucleotide of claim 1). It is moreover unlikely that a polypeptide of 10 amino acids may have the technical effect claimed, i.e., be an auxin transport protein. This also applies to claims 12 and 13.

2.3 The method claims 16, 19-23 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem instead of defining the subject-matter in terms of technical features.

Concerning claim 20, the Applicant fails to provide any data concerning null mutants of the claimed polynucleotides, thus leaving the skilled person guessing which phenotype he/she should be looking for.

1✓

PATENT COOPERATION TREATY

REC'D 09 AUG 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BB1355 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/12061	International filing date *(day/month/year)* 03/05/2000	Priority date *(day/month/year)* 07/05/1999
International Patent Classification (IPC) or national classification and IPC C12N15/29		
Applicant E.I. DU PONT DE NEMOURS AND COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/11/2000	07.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Rojo Romeo, E Telephone No. +49 89 2399 7321

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/12061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-37 as originally filed

Claims, No.:

1-24 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-45, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-24 (partially).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-24 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   - ☐ complied with.
   - ☒ not complied with for the following reasons:
     see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-24 (partially).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24 (partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24 (partially)
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-24 (partially)

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of this report

Since the applicant did not file an answer to the Written Opinion, the present International Preliminary Examination Report is based on said opinion.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability.

Since the Applicant failed to pay additional search fees, claims 1-24 were only searched as far as they concerned SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 ,12, 13, 14, 47 and 48.

Examination is thus carried out on claims 1-24 (partially as far as they concern these sequences.

Re Item IV

Lack of unity of invention

According to Rule 13 PCT an application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept, i.e. having at least one common technical feature defining a contribution over the known prior art.

The International Preliminary Examination Authority agrees with the objection raised for lack of unity by the International Search Authority. The ISA found that the present application concerns 4 groups of inventions:

invention 1 (claims 1-24, partially)

An isolated polynucleotide derived from maize selected from the group of: a nucleotide sequence encoding a polypeptide of at least 30 amino acids that is at least 85% identical to the sequence set forth in SEQ ID NO: 6, of at least 50 amino acids being at least 85% identical to SEQ ID NO: 12, of at least 50 amino acids being at least 90% identical to SEQ ID NO: 8, of at least 1000 amino acids being at least 90%identical to SEQ ID NO: 2, of at least 150 amino acids being at least 95% identical to SEQ ID NO: 4, of at least 350 amino acids being at least 95% identical to SEQ ID O: 10, of at least 200 amino acids being at least 80% identical to SEQ ID NO: 14, or of at least 250 amino acids being at least 95% identical to SEQ ID NO: 48, or a polynucleotide sequence complementary thereto.

Said polynucleotide sequences comprising the sequences selected from SEQ ID NO: 1,

3, 5, 7, 9, 11, 13, 47, being DNA or RNA.
Chimeric genes, transformed host cells, proteins derived therefrom.
Use of said sequences in a method for modulating root development in plants, and for diagnostic screening purposes. Products derived from said diagnostic screening studies.

invention 2 (claims 1-24, partially)
idem for SEQ ID NO: 15-20, derived from rice.

invention 3 (claims 1-24, partially)
idem for SEQ ID NO: 21-34, derived from soybean.

invention 4 (claims 1-24, partially)
idem for SEQ ID NO: 35-42, 45, 46, derived from wheat.

Plant auxin transport proteins have been described and cloned in the prior art (see e.g. D1, D2, D5, D6), and used in plant genetic engineering.

In the light of the prior art, the problem underlying the present application is the provision of alternative plant auxin transport protein encoding sequences.
The solution to this problem as provided herein are the following:
1. solution: an isolated polynucleotide from maize selected from the group as defined and listed, or a polynucleotide sequence complementary thereto.
Chimeric genes, transformed host cells, proteins derived therefrom.
Use of said sequences in a method for modulating root development in plants, and for diagnostic screening purposes. Products derived from said diagnostic screening studies.
2. solution: idem for SEQ ID NO: 15-20, derived from rice.
3. solution: idem for SEQ ID NO: 21-34, derived form soybean.
4. solution: idem for SEQ ID NO: 35-42, 45, 46, derived from wheat.

Due to the prior art disclosing auxin transport proteins from plant sources, due to the essential difference of the primary structure of the claimed nucleic acid molecules and proteins of the present application, and due to the fact that no other technical features could be distinguished, which in the light of the prior art could be regarded as special technical features, the ISA was of the opinion that there was no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule

13.1 PCT. Consequently, there is lack of unity.

Since the Applicant failed to pay additional search fees, only the first group of inventions is considered for the examination.

The Applicant's attention is drawn to the fact that the first group of invention lacks unity and will be split, at the regional phase, into as may independent inventions as there are sequences claimed. Indeed, the common inventive concept linking the claims as far as they concern invention 1 is that the claimed nucleotide and polypeptide molecules are of maize origin. Nucleic acids and proteins of maize origin are known from prior art (e.g. D3, D4). Thus, this common inventive concept does not exist.

__Re Item V__

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents cited in the International Search Report:

D1: DATABASE EBI [Online] AC O81215, 1 November 1998 (1998-11-01) LUSCHNIG ET AL.: 'Rice EIR1 homologue REH1' XP002146833

D2: LUSCHNIG C ET AL: 'EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana' GENES AND DEVELOPMENT,US,COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, vol. 12, no. 14, 15 July 1998 (1998-07-15), pages 2175-2187, XP002116368 ISSN: 0890-9369

D3: SCHWOB E ET AL: 'MOLECULAR ANALYSIS OF THREE MAIZE 22 KDA AUXIN-BINDING PROTEIN GENES - TRANSIENT PROMOTER EXPRESSION AND REGULATORY REGIONS' PLANT JOURNAL,GB,BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, vol. 4, no. 3, 1993, pages 423-432, XP002024567 ISSN: 0960-7412

D4: ZETTL R ET AL: '5' AZIDO-3 6-TRITIATED-1-NAPHTHYLPHTHALAMIC ACID A PHOTOACTIVATABLE PROBE FOR NAPHTHYLPHTHALAMIC ACID RECEPTOR PROTEINS FROM HIGHER PLANTS IDENTIFICATION OF A 23-KDA PROTEIN FROM MAIZE COLEOPTILE PLASMA MEMBRANES' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 2, 1992, pages 480-484, XP002146831 1992 ISSN: 0027-8424

D5: GÄLWEILER L. ET AL.: 'Regulation of polar auxin transport by AtPIN1 in

Arabidopsis vascular tissue' SCIENCE, vol. 282, December 1998 (1998-12), pages 2226-2230, XP002146832

D6: EP-A-0 814 161 (MAX PLANCK GESELLSCHAFT) 29 December 1997 (1997-12-29) cited in the application

1. Novelty (Art. 33(2) PCT)

None of the documents cited in the International Search Report discloses the claimed subject-matter. The current set of claims is thus considered novel over these documents, given that the clarity problems set below are relieved.

2. Inventive step (Art. 33(3) PCT)

2.1 As mentioned above, auxin transport proteins are known from prior art. The problem underlying the current application is the provision of alternative auxin transport proteins from maize. The solution provided by the current application is the provision of the DNA molecules of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, and 47. Although the application discloses the nucleotide sequence of these open reading frames (ORF), these sequences appear to be the "best guess" ORFs identifiable as putative auxin transport proteins. The Applicant fails, however, to provide tangible evidence of the expression of these proteins, of their identity as auxin transport proteins and of their role (the auxin transport proteins were shown to have varied phenotypes if disrupted). Indeed, these sequences appear to be the result of mere computer work (see examples) by sequence comparison for the identification of the homologs of the already known auxin transport proteins: for the person skilled in the art, the review of a gene database or an EST database as described in the present application (examples) belongs to standard techniques and does not involve any inventive activity. Hence, in the absence of experimental data, the DNA molecules and proteins putatively encoded claimed constitute "compounds" with no known technically useful property. In this case, any prior art compound identifiable as an auxin transport protein (see D1-D6), regardless of its technical properties, is equally suitable as the starting point for making structural modifications and may be considered to represent the closest prior art. Without the concomitant need to provide any particular technical effect, for the skilled person any putative auxin transport protein (see e.g. D4) may provide an equally obvious solution.

Consequently, the solution provided by the current application (SEQ ID NO: 1, 3, 5,

7, 9, 11, 13, and 47 and corresponding amino acid sequences 2, 4, 6, 8, 10, 12, 14, and 48) cannot be considered inventive.

Embodiments of these sequences and methods claimed are considered to be obvious to the skilled person and thus, not inventive.

The Applicant's attention is drawn to the fact that D4 also describes a maize protein which may be involved in auxin transport and thus gives a clear hint for auxin transport proteins to be found in this plant. Thus, also for this reason, the present application cannot be considered inventive over prior art since the skilled person would be prompted to look for auxin transport proteins in maize.

Consequently, claims 1-24 lack inventive step.

3. Industrial applicability (Art. 33(4) PCT)
The Applicant's attention is drawn to the fact that the claimed DNA and polypeptide molecules claimed lack technical effects. The putative functions of these different molecules were assigned by computer sequence analysis without providing actual proof of the function of any of these molecules. For the EPO, compounds without function are not considered to have an industrial applicability. Thus, the present set of claims has no industrial applicability.

Re Item VIII

Certain observations on the international application

1. Clarity (Art. 6 PCT)

1.1 Claim 1 is directed to "an" isolated polynucleotide encoding "a" polypeptide having homology to the claimed SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 and 48 without any technical effect since no function is indicated

1.2 Claim 11 is directed to a method of selecting an isolated polypeptide that affects the level of expression of "any" polypeptide. It is also unclear whether this should be a direct effect (peptide encoded by the 30 nucleotides?) or an indirect effect of said polypeptide on any other polypeptide.

The same objection applies to claims 14 and 15, since the polypeptide encoded by

the nucleic acid to clone has no particular function.

1.3 Concerning claim 24, the lack of reference to a particular sequence leads to a method for modulating root development in a plant using any auxin transport protein known, not only those claimed in the present specification. Consequently, an objection for lack of novelty may also arise.

2. Support by specification (Art. 6 PCT), in combination with Art. 5 PCT (complete and enabling disclosure)

2.1 Art. 6 PCT requires the claims to be fully supported by the description, i.e. the claims must be formally and technically supported by the description. The IPEA considers that the present description actually only discloses polynucleotides and bacterial host cells transformed with this polynucleotide (library clones). The description refers to the predicted proteins based only on the possible ORF of said polynucleotide. However, there is no real characterization of any protein (N-ter or C-ter sequence, complete amino acid sequence, etc), let alone of an "isolated polypeptide". Thus, the IPEA considers that such a subject-matter has no technical support. Furthermore, the fact that these putative polypeptides have a certain homology to other known proteins is far away from an actual characterization of any real "biological activity". Thus the IPEA considers that claim10, and dependent claim18 which refer to the putative amino acid sequence of proteins which have not been actually disclosed or well characterized and which have not been shown to have the claimed activity, are not supported by the description (Art. 6 PCT).

2.2 The present application is silent as far as the method claimed in claim 11 is concerned. Indeed, no basis is given for the influence of a polynucleotide of at least 30 nucleotides (derived from the polynucleotide of claim 1). It is moreover unlikely that a polypeptide of 10 amino acids may have the technical effect claimed, i.e., be an auxin transport protein. This also applies to claims 12 and 13.

2.3 The method claims 16, 19-23 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem instead of defining the subject-matter in terms of technical features.

Concerning claim 20, the Applicant fails to provide any data concerning null mutants of the claimed polynucleotides, thus leaving the skilled person guessing which phenotype he/she should be looking for.

TMR BB1355 MAR 27 2001

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(19) World Intellectual Property Organization
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(21) International Application Number: PCT/US00/12061

(22) International Filing Date: 3 May 2000 (03.05.2000)

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(30) Priority Data:
60/133,040 7 May 1999 (07.05.1999) US

(71) Applicants *(for all designated States except US)*: **E. I. DU PONT DE NEMOURS AND COMPANY** [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). **PIONEER HI-BRED INTERNATIONAL, INC.** [US/US]; 7100 N.W. 62nd Avenue, Johnston, IA 50131 (US).

(72) Inventors; and
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(74) Agent: **GEIGER, Kathleen, W.**; E.I. du Pont de Nemours and Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,

[Continued on next page]

(54) Title: AUXIN TRANSPORT PROTEINS


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                 ***  * * *  * *******  ***** ** ** ************ ***********
SEQ ID NO:14     MITALDLYHVLTAVVPLYVAMTLAYGSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFIS
SEQ ID NO:30     MITLTDFYHVMTAMVPLYVAMILAYGSVKWWKIFSPDQCSGINRFVALFAVPLLSFHFIA
SEQ ID NO:34     MITGKDIYDVFAAIVPLYVAMILAYGSVRWWKIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:38     MITGKDIYDVLAAVVPLYVAMFMAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:43     MITGKDMYDVLAAMVPLYVAMILAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:44     MITAADFYHVMTAMVPLYVAMILAYGSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFIA
                 1                                                          60

                  * *  *   * *** ***   *  *      *      *         * * ******
SEQ ID NO:14     TNDPFAMNLRFLAADTLQKVAVLALLALASRGLSSPRALG---------LDWSITLFSLS
SEQ ID NO:30     SNNPYEMNLRFLAADTLQKIIILVLLAVW----SNITKRG--------CLEWAITLFSLS
SEQ ID NO:34     SNDPYAMNYHFIAADCLQKVVILGALFLWNT----FTKHG--------SLDWTITLFSLS
SEQ ID NO:38     TNDPYAMDYRFLAADSLQKLVILAALAVWHNVLSRYRCRGGTEAGEASSLDWTITLFSLA
SEQ ID NO:43     SNDPYAMNYHFLAADSLQKVVILAALFLWQA----FSRRG--------SLEWMITLFSLS
SEQ ID NO:44     ANNPYAMNLRFLAADSLQKVIVLSLLFLW----CKLSRNG--------SLDWTITLFSLS
                61                                                         120

                 *************  ***  * * ****  ****  ******** ** * *  *   ***
SEQ ID NO:14     TLPNTLVMGIPLLRGMYGASSAGTLMVQVVVLQCIIWYTLMLFLFEYRAARALVLDQFPD
SEQ ID NO:30     TLPNTLVMGIPLLKGMYGDFS-GSLMVQIVVLQCIIWYTLMLFLFEFRGARMLISEQFP-
SEQ ID NO:34     TLPNTLVMGIPLLKAMYGDFS-GSLMVQIVVLQSVIWYTLMLFMFEYRGAKLLITEQFP-
SEQ ID NO:38     TLPNTLVMGIPLLRAMYGDFS-GSLMVQIVVLQSVIWYTLMLFLFEYRGAKALISEQFPP
SEQ ID NO:43     TLPNTLVMGIPLLRAMYGDFS-GNLMVQIVVLQSIIWYTLMLFLFEFRGAKLLISEQFP-
SEQ ID NO:44     TLPNTLVMGIPLLKGMYGNFS-GDLMVQIVVLQCIIWYILMLFLFEYRGAKLLISEQFP-
                121                                                        180
```

(57) **Abstract:** This invention relates to an isolated nucleic acid fragment encoding an auxin transport protein. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the auxin transport protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the auxin transport protein in a transformed host cell. The present invention also relates to methods using the auxin transport protein in modulating root development, and in discovering compounds with potential herbicidal activity.

WO 00/68389 A3

MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

(88) Date of publication of the international search report: 15 March 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PCT

WORLD INTELLECTUAL PROPERTY ORGANI[redacted]N
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/29, 15/82, 5/10, C12Q 1/68, C07K 14/415	A2	(11) International Publication Number: WO 00/68389 (43) International Publication Date: 16 November 2000 (16.11.00)

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(30) Priority Data:
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(74) Agent: GEIGER, Kathleen, W.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: AUXIN TRANSPORT PROTEINS

```
                  ***  * * *  * *******  ***** ** ** ************ ***********
SEQ ID NO:14      MITALDLYHVLTAVVPLYVAMTLAYGSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFIS
SEQ ID NO:30      MITLTDFYHVMTAMVPLYVAMILAYGSVKWWKIFSPDQCSGINRFVALFAVPLLSFHFIA
SEQ ID NO:34      MITGKDIYDVFAAIVPLYVAMILAYGSVRWWKIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:38      MITGKDIYDVLAAVVPLYVAMFMAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:43      MITGKDMYDVLAAMVPLYVAMILAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:44      MITAADFYHVMTAMVPLYVAMILAYGSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFIA
                  1                                                          60

                   * *  *   * *** ***   *  *      *      *         * * ******
SEQ ID NO:14      TNDPFAMNLRFLAADTLQKVAVLALLALASRGLSSPRALG---------LDWSITLFSLS
SEQ ID NO:30      SNNPYEMNLRFLAADTLQKIIILVLLAVW----SNITKRG--------CLEWAITLFSLS
SEQ ID NO:34      SNDPYAMNYHFIAADCLQKVVILGALFLWNT----FTKHG--------SLDWTITLFSLS
SEQ ID NO:38      TNDPYAMDYRFLAADSLQKLVILAALAVWHNVLSRYRCRGGTEAGEASSLDWTITLFSLA
SEQ ID NO:43      SNDPYAMNYHFLAADSLQKVVILAALFLWQA----FSRRG--------SLEWMITLFSLS
SEQ ID NO:44      ANNPYAMNLRFLAADSLQKVIVLSLLFLW----CKLSRNG--------SLDWTITLFSLS
                 61                                                         120

                  *************  ***  * * **** ****  ******** ** * *  *   ***
SEQ ID NO:14      TLPNTLVMGIPLLRGMYGASSAGTLMVQVVVLQCIIWYTLMLFLFEYRAARALVLDQFPD
SEQ ID NO:30      TLPNTLVMGIPLLKGMYGDFS-GSLMVQIVVLQCIIWYTLMLFLFEFRGARMLISEQFP-
SEQ ID NO:34      TLPNTLVMGIPLLKAMYGDFS-GSLMVQIVVLQSVIWYTLMLFMFEYRGAKLLITEQFP-
SEQ ID NO:38      TLPNTLVMGIPLLRAMYGDFS-GSLMVQIVVLQSVIWYTLMLFLFEYRGAKALISEQFPP
SEQ ID NO:43      TLPNTLVMGIPLLRAMYGDFS-GNLMVQIVVLQSIIWYTLMLFLFEFRGAKLLISEQFP-
SEQ ID NO:44      TLPNTLVMGIPLLKGMYGNFS-GDLMVQIVVLQCIIWYILMLFLFEYRGAKLLISEQFP-
                121                                                         180
```

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding an auxin transport protein. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the auxin transport protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the auxin transport protein in a transformed host cell. The present invention also relates to methods using the auxin transport protein in modulating root development, and in discovering compounds with potential herbicidal activity.

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CM	Cameroon			PL	Poland		
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CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/12061

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/29 C12N15/82 C12N5/10 C12Q1/68 C07K14/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE EBI [Online] AC 081215, 1 November 1998 (1998-11-01) LUSCHNIG ET AL.: "Rice EIR1 homologue REH1" XP002146833 abstract	1-24
A	LUSCHNIG C ET AL: "EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana" GENES AND DEVELOPMENT, US, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, vol. 12, no. 14, 15 July 1998 (1998-07-15), pages 2175-2187, XP002116368 ISSN: 0890-9369 the whole document	1-24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 September 2000	16. 01. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHWOB E ET AL: "MOLECULAR ANALYSIS OF THREE MAIZE 22 KDA AUXIN-BINDING PROTEIN GENES - TRANSIENT PROMOTER EXPRESSION AND REGULATORY REGIONS" PLANT JOURNAL,GB,BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, vol. 4, no. 3, 1993, pages 423-432, XP002024567 ISSN: 0960-7412 the whole document ---	1-24
A	ZETTL R ET AL: "5' AZIDO-3 6-TRITIATED-1-NAPHTHYLPHTHALAMIC ACID A PHOTOACTIVATABLE PROBE FOR NAPHTHYLPHTHALAMIC ACID RECEPTOR PROTEINS FROM HIGHER PLANTS IDENTIFICATION OF A 23-KDA PROTEIN FROM MAIZE COLEOPTILE PLASMA MEMBRANES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 2, 1992, pages 480-484, XP002146831 1992 ISSN: 0027-8424 the whole document ---	1-24
A	GÄLWEILER L. ET AL.: "Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue" SCIENCE, vol. 282, December 1998 (1998-12), pages 2226-2230, XP002146832 the whole document ---	1-24
A	EP 0 814 161 A (MAX PLANCK GESELLSCHAFT) 29 December 1997 (1997-12-29) cited in the application the whole document -----	1-24

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/12061

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-24, partially.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24 partially

An isolated polynucleotide derived from maize selected from the group of: a nucleotide sequence encoding a polypeptide of at least 30 amino acids that is at least 85% identical to the sequence set forth in SEQ ID NO:6, of at least 50 amino acids being at least 85% identical to SEQ ID NO:12, of at least 50 amino acids being at least 90% identical to SEQ ID NO:8, of at least 100 amino acids being at least 90% identical to SEQ ID NO:2, of at least 150 amino acids being at least 95% identical to SEQ ID NO:4, of at least 350 amino acids being at least 95% identical to SEQ ID NO:10, of at least 200 amino acids being at least 80% identical to SEQ ID NO:14, or of at least 250 amino acids being at least 90% identical to SEQ ID NO:48, or a polynucleotide sequence complementary thereto.
Said polynucleotide sequences comprising the sequences selected from SEQ ID NO:1,3,5,7,9,11,13,47, being DNA or RNA.
Chimeric genes, transformed host cells, proteins derived therefrom.
Use of said sequences in a method for modulating root development in plants, and for diagnostic screening purposes. Products derived from said diagnostic screening studies.

2. Claims: 1-24 partially

idem for SEQ ID NO:15-20 derived from rice

3. Claims: 1-24 partially

idem for SEQ ID NO:21-34 derived from soybean

4. Claims: 1-24 partially

idem for SEQ ID NO:35-42,45,46 derived from wheat

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/12061

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0814161 A	29-12-1997	AU 3539597 A	14-01-1998
		CA 2259125 A	31-12-1997
		WO 9749810 A	31-12-1997
		JP 2000513218 T	10-10-2000

4/PRTS

10/030884

531 R PCT/PTO PCT/US00/12861 25 OCT 2001

TITLE

AUXIN TRANSPORT PROTEINS

This application claims the benefit of U.S. Provisional Application No. 60/133,040, filed May 7, 1999.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding auxin transport proteins in plants and seeds.

BACKGROUND OF THE INVENTION

Auxins are a major class of plant hormones that influence diverse aspects of plant behavior and development including vascular tissue differentiation, apical development, tropic responses, and organ (e.g., flower, leaf) development. The term "auxin" refers to a diverse group of natural and synthetic chemical substances that are able to stimulate elongation growth in coleoptiles and many stems. Indole-3-acetic acid (IAA) is the principal auxin in higher plants, though other molecules such as 4-chloroindole-3-acetic acid and phenylacetic acid have been shown to have auxin activity. Synthetic auxins include 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D); both are commonly used as herbicides.

Distribution of auxins in concentration gradients within plant organs enables auxins to convey to cells their relative location, allowing the plants to respond accordingly to a given stimulus. A classic example that illustrates auxin action is the differential growth and curvature of etiolated coleoptiles exposed to light. It is believed that the phototropic stimulus results in a lateral redistribution of auxin in the coleoptile such that the shaded side has a higher auxin concentration than the illuminated side. With more auxin stimulating cell elongation on the shaded side, the end-result is the apparent bending of the coleoptile towards the light source.

The foregoing description underscores the importance of polar transport in auxin function. Not surprisingly, a number of genetic and physiological studies have focused on the polar auxin transport system operating in plant cells. *Arabidopsis* mutants with impaired auxin transport capabilities exhibit varying phenotypes: pin1 mutants develop naked, pin-like inflorescences with few normal flowers (Gälweiler, L. et al., (1998) *Science 282*:2226-2230), while defects in pin2 (also called eir1 and agr1) are restricted to the root, altering growth and gravitropic response (Luschnig, C. et al., (1998) *Genes Dev. 12*:2175-2187). Proteins encoded by AUX1, PIN1 and PIN2 genes which have been identified to be important for auxin transport and are putative membrane proteins that have significant homology with a number of bacterial membrane transporters (Luschnig, C. et al. *supra*; Gälweiler L. et al., (1998) *Science 282*:2226-2230; Bennett, M. J. et al., (1996)

Science 273:948-950; WO 99/63092-A1; U.S. Application No. 90/087,789; EP 0 814 161 A1), consistent with a role for these proteins in auxin transport.

Since auxin affects several aspects of plant development, and polar transport is a vital component of auxin function, it is envisioned that proteins involved in auxin polar transport may serve as potential targets for new herbicide discovery and design. Blocking of normal function of these auxin transport proteins can cause severe plant growth defects; this is supported by the phenotype of mutants where a particular auxin transport protein has been rendered nonfunctional, particularly the *Arabidopsis* pin1 mutants. In addition, since some of these auxin transport proteins have been shown to be root-specific and impact root development to a significant degree, manipulation of auxin transport proteins may be a powerful strategy for developing more robust root systems in plants, which in turn may enhance food production, especially in arid climates.

SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 30 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6; (b) a second nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40; (c) a third nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12; (d) a fourth nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24; (e) a fifth nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32; (f) a sixth nucleotide sequence encoding a polypeptide of at least 90 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42; (g) a seventh nucleotide sequence encoding a polypeptide of at least 95 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46; (h) an eighth nucleotide sequence encoding a polypeptide of at least 100 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20; (i) a ninth nucleotide sequence encoding a polypeptide of at least 100 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2; (j) a tenth nucleotide sequence encoding a polypeptide of at least 150 amino acids having at least 95%

identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4; (k) an eleventh nucleotide sequence encoding a polypeptide of at least 300 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38; (l) a twelfth nucleotide sequence encoding a polypeptide of at least 350 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10; (m) a thirteenth nucleotide sequence encoding a polypeptide of at least 400 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30; (n) a fourteenth nucleotide sequence encoding a polypeptide of at least 500 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34; (o) a fifteenth nucleotide sequence encoding a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; (p) a sixteenth nucleotide sequence encoding a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48; and (q) a seventeenth nucleotide sequence comprising the complement of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), or (p).

In a second embodiment, it is preferred that the isolated polynucleotide of the claimed invention comprises a first nucleotide sequence which comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and the complement of such nucleotide sequences.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting a compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

In a seventh embodiment, the invention concerns an auxin transport polypeptide selected from the group consisting of: (a) a polypeptide of at least 30 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6; (b) a polypeptide of at least 50 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40; (c) a polypeptide of at least 50 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12; (d) a polypeptide of at least 50 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24; (e) a polypeptide of at least 50 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32; (f) a polypeptide of at least 90 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42; (g) a polypeptide of at least 95 amino acids having at least 95% identity basaed on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46; (h) a polypeptide of at least 100 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20; (i) a polypeptide of at least 100 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2; (j) a polypeptide of at least 150 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4; (k) a polypeptide of at least 300 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38; (l) a polypeptide of at least 350 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10; (m) a polypeptide of at least 400 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30; (n) a polypeptide of at least 500 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34; (o) a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; and (p) a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48.

In an eighth embodiment, the invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of an auxin transport polypeptide or enzyme activity in a host cell, preferably a plant cell, the method comprising the steps of: (a) constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; (b) introducing the isolated polynucleotide or the chimeric gene into a host cell; (c) measuring the level of the auxin transport polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and (d) comparing the level of the auxin transport polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of the auxin transport polypeptide or enzyme activity in the host cell that does not contain the isolated polynucleotide.

In a ninth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of an auxin transport polypeptide, preferably a plant auxin transport polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an auxin transport polypeptide amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding an auxin transport polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide or isolated polypeptide of the present invention.

In a twelfth embodiment, this invention concerns a method for positive selection of a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or a construct of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot, under conditions which allow expression of the auxin transport polypeptide polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an auxin transport protein, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a

nucleic acid fragment encoding an auxin transport polypeptide, operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the encoded auxin transport protein in the transformed host cell; (c) optionally purifying the auxin transport polypeptide expressed by the transformed host cell; (d) treating the auxin transport polypeptide with a compound to be tested; and (e) comparing the activity of the auxin transport polypeptide that has been treated with a test compound to the activity of an untreated auxin transport polypeptide, thereby selecting compounds with potential for inhibitory activity.

In a further embodiment, the instant invention concerns a method of modulating expression of an auxin transport protein in a plant, comprising the steps of: (a) transforming a plant cell with a nucleic acid fragment encoding the auxin transport protein operably linked in sense or antisense orientation to a promoter; and (b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the nucleic acid for a time sufficient to modulate expression of the nucleic acid fragment in the plant compared to a corresponding non-transformed plant, thereby resulting in at least one of the following: a more robust root system, an altered root angle, or redirected root growth.

BRIEF DESCRIPTION OF THE DRAWING AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description, the accompanying drawing and Sequence Listing which form a part of this application.

Figure 1 depicts the amino acid sequence alignment between the auxin transport protein encoded by the nucleotide sequences derived from the corn clone p0119.cmtn124r (SEQ ID NO:14), soybean clone sfl1.pk131.g9 (SEQ ID NO:30), soybean clone src3c.pk026.o11 (SEQ ID NO:34), and wheat clone wdk1c.pk008.g12 (SEQ ID NO:38), the auxin transport protein EIR1 from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 3377507; SEQ ID NO:43), and the auxin transport protein AtPIN1 from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 4151319; SEQ ID NO:44). Amino acids which are conserved among all and at least two sequences with an amino acid at that position are indicated with an asterisk (*). Dashes are used by the program to maximize alignment of the sequences.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. Table 1 also identifies the cDNA clones as individual ESTs ("EST"), sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding at a

minimum the mature protein derived from an EST, FIS, a contig, or an FIS and PCR ("CGS"). Nucleotide SEQ ID NOs:5, 7, 11, 17, 23, 27, 31, 35, and 41 correspond to nucleotide SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, and 17, respectively, presented in U.S. Provisional Application No. 60/133,040, filed May 7, 1999. Amino acid SEQ ID NOs:6, 8, 12, 18, 24, 28, 32, 36, and 42 correspond to amino acid SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, and 18, respectively, presented in U.S. Provisional Application No. 60/133,040, filed May 7, 1999. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Auxin Transport Proteins

Protein (Plant Source)	Clone Designation	Status	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Auxin Transport Protein (Corn)	ceb1.pk0082.a5	EST	1	2
Auxin Transport Protein (Corn)	Contig of: cr1.pk0022.a4 cr1n.pk0033.e3 csi1n.pk0045.a5 csi1n.pk0050.d5 p0005.cbmej72r p0041.crtba02r	Contig	3	4
Auxin Transport Protein (Corn)	p0016.ctsag12r	EST	5	6
Auxin Transport Protein (Corn)	Contig of: p0097.cqrai63r p0094.csssh17r	Contig	7	8
Auxin Transport Protein (Corn)	p0094.csssh17r	FIS	9	10
Auxin Transport Protein (Corn)	p0119.cmtnl24r	EST	11	12
Auxin Transport Protein (Corn)	cil1c.pk001.b7	FIS	47	48
Auxin Transport Protein (Corn)	p0119.cmtnl24r	CGS	13	14
Auxin Transport Protein (Rice)	rr1.pk0019.c4	EST	15	16
Auxin Transport Protein (Rice)	rsl1n.pk003.n3	EST	17	18
Auxin Transport Protein (Rice)	rsl1n.pk003.n3	FIS	19	20
Auxin Transport Protein (Soybean)	scr1c.pk003.g7	FIS	21	22

Protein (Plant Source)	Clone Designation	Status	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Auxin Transport Protein (Soybean)	sdp4c.pk003.h2	EST	23	24
Auxin Transport Protein (Soybean)	sdp4c.pk003.h2	FIS	25	26
Auxin Transport Protein (Soybean)	sfl1.pk131.g9	EST	27	28
Auxin Transport Protein (Soybean)	sfl1.pk131.g9(FIS)	CGS	29	30
Auxin Transport Protein (Soybean)	src3c.pk026.o11	EST	31	32
Auxin Transport Protein (Soybean)	src3c.pk026.o11(FIS)	CGS	33	34
Auxin Transport Protein (Wheat)	wdk1c.pk008.g12	EST	35	36
Auxin Transport Protein (Wheat)	wdk1c.pk008.g12(FIS)	CGS	37	38
Auxin Transport Protein (Wheat)	wdr1f.pk001.g9	EST	39	40
Auxin Transport Protein (Wheat)	wle1n.pk0109.h1	EST	41	42
Auxin Transport Protein (Wheat)	wle1n.pk0109.h1	FIS	45	46

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res. 13*:3021-3030 (1985) and in the *Biochemical J. 219 (No. 2)*:345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably

one of at least 30 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, 47 or the complement of such sequences.

The term "isolated polynucleotide" refers to a polynucleotide that is substantially free from other nucleic acid sequences, such as and not limited to other chromosomal and extrachromosomal DNA and RNA. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment

representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of an auxin transport polypeptide in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a virus or in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise the steps of: constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; introducing the isolated polynucleotide or the chimeric gene into a host cell; measuring the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or

DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.).. Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 30 or 50 amino acids, preferably at least 90 or 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250, 300, 350, 400 or 500 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* *5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-

based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan

appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences

have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

"3' Non-coding sequences" refers to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptides by the cell. "cDNA" refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. "Sense RNA" refers to an RNA transcript that includes the mRNA and can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. "Expression" may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. In the context of this disclosure, a number of terms shall be utilized. The terms "protein" and "polypeptide" are used interchangeably herein. Each protein or polypeptide has a unique function.

"Altered levels" or "altered expression" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Null mutant" refers to a host cell which either lacks the expression of a certain polypeptide or expresses a polypeptide which is inactive or does not have any detectable expected enzymatic function.

"Mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor protein" refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the

transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London) 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Flevin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

"PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 30 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6; (b) a second nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40; (c) a third nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12; (d) a fourth nucleotide sequence encoding a polypeptide of at least 50 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24; (e) a fifth nucleotide sequence encoding

a polypeptide of at least 50 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32; (f) a sixth nucleotide sequence encoding a polypeptide of at least 90 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42; (g) a seventh nucleotide sequence encoding a polypeptide of at least 95 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46; (h) an eighth nucleotide sequence encoding a polypeptide of at least 100 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20; (i) a ninth nucleotide sequence encoding a polypeptide of at least 100 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2; (j) a tenth nucleotide sequence encoding a polypeptide of at least 150 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4; (k) an eleventh nucleotide sequence encoding a polypeptide of at least 300 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38; (l) a twelfth nucleotide sequence encoding a polypeptide of at least 350 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10; (m) a thirteenth nucleotide sequence encoding a polypeptide of at least 400 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30; (n) a fourteenth nucleotide sequence encoding a polypeptide of at least 500 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34; (o) a fifteenth nucleotide sequence encoding a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; (p) a sixteenth nucleotide sequence encoding a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48; and (q) a seventeenth nucleotide sequence comprising the complement of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o) or (p).

Preferably, the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.

Nucleic acid fragments encoding at least a substantial portion of several auxin transport proteins have been isolated and identified by comparison of random plant cDNA

sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other auxin transport polypeptides, either as cDNAs or genomic DNAs, could be isolated directly by using all or a substantial portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequence(s) can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one

of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of an auxin transport polypeptide, preferably a substantial portion of a plant auxin transport polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an auxin transport polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing substantial portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of auxin efflux in those cells. In addition, since some of these auxin transport proteins may be root-specific and impact root development to a significant degree, these auxin transport proteins may lead to novel strategies for developing transgenic plants with more robust root systems, which may enhance food production, especially in arid climates. The nucleic acid fragments of the instant invention may also be used to regulate root angle, and thus modify plant susceptibility to root lodging, root angle being a determinant of lodging susceptibility. Modified root gravitropic responses (as mediated by manipulation of the nucleic acid fragments of the instant invention) would also be useful for redirecting root growth (by

inhibiting gravitropism in short durations) for soil remediation projects and alleviate soil erosion problems. Roots may also be made to grow deeper beyond the top layers of the soil, reducing root tip damage caused by insect feeding and possibly generating a root system that extends downward rather than laterally into neighboring root zones, thus minimizing competition for nutrients among different root systems, making planting at higher densities a possibility. The auxin transport proteins disclosed herein may also be engineered to transport other compounds into and/or out of the plant, for example, such as into storage compartments or into media for harvesting.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide (or chimeric gene) may be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.100*:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric

gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

In another embodiment, the present invention concerns an auxin transport polypeptide selected from the group consisting of: (a) a polypeptide of at least 30 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6; (b) a polypeptide of at least 50 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40; (c) a polypeptide of at

least 50 amino acids having at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12; (d) a polypeptide of at least 50 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24; (e) a polypeptide of at least 50 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32; (f) a polypeptide of at least 90 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42; (g) a polypeptide of at least 95 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46; (h) a polypeptide of at least 100 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20; (i) a polypeptide of at least 100 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2; (j) a polypeptide of at least 150 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4; (k) a polypeptide of at least 300 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38; (l) a polypeptide of at least 350 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10; (m) a polypeptide of at least 400 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30; (n) a polypeptide of at least 500 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34; (o) a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; (p) a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded auxin transport protein.

An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

Additionally, the instant auxin transport proteins can be used as a target to facilitate design and/or identification of inhibitors of these proteins that may be useful as herbicides. This is desirable because the auxin transport proteins described herein are essential components of the polar transport system involved in auxin redistribution and hence auxin function. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth. Thus, the instant auxin transport proteins could be appropriate for new herbicide discovery and design.

The present invention further provides a method for modulating (i.e., increasing or decreasing) the concentration or composition of the polypeptides of the present invention in a plant or part thereof. Modulation of the polypeptides can be effected by increasing or decreasing the concentration and/or the composition of the polypeptides in a plant. The method comprises transforming a plant cell with a construct comprising a nucleic acid fragment of the present invention to obtain a transformed plant cell, growing the transformed plant cell under plant forming conditions, and expressing the nucleic acid fragment in the plant for a time sufficient to modulate concentration and/or composition of the polypeptides in the plant or plant part.

In some embodiments, the content and/or composition of polypeptides of the present invention in a plant may be modulated by altering, *in vivo* or *in vitro,* the promoter of a non-isolated gene of the present invention to up- or down-regulate gene expression. In some embodiments, the coding regions of native genes of the present invention can be altered via substitution, addition, insertion, or deletion to decrease activity of the encoded enzyme. See, e.g., Kmiec, U.S. Patent No. 5,565,350; Zarling *et al.*, PCT/US93/03868.

In some embodiments, an isolated nucleic acid fragment (e.g., a vector) comprising a promoter sequence is transfected into a plant cell. Subsequently, a plant cell comprising the isolated nucleic acid is selected for by means known to those of skill in the art such as, but not limited to, Southern blot, DNA sequencing, or PCR analysis using primers specific to the promoter and to the nucleic acid and detecting amplicons produced therefrom. A plant or plant part altered or modified by the foregoing embodiments is grown under plant forming conditions for a time sufficient to modulate the concentration and/or composition of polypeptides of the present invention in the plant. Plant forming conditions are well known in the art.

In general, concentration of the polypeptides is increased or decreased by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% relative to a native control plant, plant part, or cell lacking the aforementioned transgene. Modulation in the present invention may occur during and/or subsequent to growth of the plant to the desired stage of development.

Modulating nucleic acid expression temporally and/or in particular tissues can be controlled by employing the appropriate promoter operably linked to a nucleic acid fragment of the present invention in, for example, sense or antisense orientation as discussed in greater detail above. Induction of expression of a nucleic acid fragment of the present invention can also be controlled by exogenous administration of an effective amount of inducing compound. Inducible promoters and inducing compounds that activate expression from these promoters are well known in the art.

Examples of inducible promoters are the Adh1 promoter which is inducible by hypoxia or cold stress, the Hsp70 promoter which is inducible by heat stress, and the PPDK promoter which is inducible by light. Also useful are promoters which are chemically inducible.

Examples of promoters under developmental control include promoters that initiate transcription preferentially in certain tissues such as leaves, roots, fruit, seeds, or flowers. An exemplary promoter is the anther specific promoter 5126 (U.S. Patent Nos. 5,689,049 and 5,689,051). Examples of seed-preferred promoters include, but are not limited to, 27 kD gamma zein promoter and waxy promoter (Boronat et al. (1986) *Plant Sci. 47*:95-102; Reina et al. (1990) *Nucleic Acids Res. 18(21)*:6426; Kloesgen et al. (1986) *Mol. Gen. Genet. 203*:237-244). Promoters that are expressed in the embryo, pericarp, and endosperm are disclosed in US applications Serial Nos. 60/097,233 filed August 20, 1998 and 60/098,230 filed August 28, 1998. The disclosures of each of these are incorporated herein by reference in their entirety.

Either heterologous or non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These promoters can also be used, for example, in chimeric genes to drive expression of antisense nucleic acids to reduce, increase, or alter concentration and/or composition of the proteins of the present invention in a desired tissue.

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted

and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter 4*:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet. 7*:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res. 5*:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med. 11*:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics 16*:325-332), allele-specific ligation (Landegren et al. (1988) *Science 241*:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res. 18*:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet. 7*:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res. 17*:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA 86*:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA 92*:8149-8153; Bensen et al. (1995) *Plant Cell 7*:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid

fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptide. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptide can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) tissues were prepared. The characteristics of the libraries are described below. Corn developmental stages are explained in the publication "How a Corn Plant Develops" from the Iowa State University Coop. Ext. Service Special Report No. 48 reprinted June 1993.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean, and Wheat

Library	Tissue	Clone
cebl	Corn Embryo 10 to 11 Days After Pollination	cebl.pk0082.a5
cillc	Corn (EB90) Pooled Immature Leaf Tissue at V4, V6 and V8	cillc.pk001.b7
crl	Corn Root From 7 Day Old Seedlings	crl.pk0022.a4
crln	Corn Root From 7 Day Old Seedlings*	crln.pk0033.e3
csiln	Corn Silk*	csiln.pk0045.a5 csiln.pk0050.d5
p0005	Corn Immature Ear	p0005.cbmej72r
p0016	Corn Tassel Shoot, Pooled, 0.1-1.4 cm	p0016.ctsag12r
p0041	Corn Root Tip Smaller Than 5 mm in Length, Four Days After Imbibition	p0041.crtba02r
p0094	Corn Leaf Collars for the Ear Leaf (EL), screened 1 and the Next Leaf Above and Below the EL; Growth Conditions: Field; Control or Untreated Tissues	p0094.csssh17r
p0097	Corn V9 Whorl Section (7 cm) From Plant Infected Four Times With European Corn Borer	p0097.cqrai63r
p0119	Corn V12-Stage Ear Shoot With Husk, Night Harvested*	p0119.cmtnl24r
rrl	Rice Root of Two Week Old Developing Seedling	rrl.pk0019.c4
rslln	Rice 15-Day-Old Seedling*	rslln.pk003.n3
scrlc	Soybean Embryogenic Suspension Culture Subjected to 4 Vacuum Cycles and Collected 12 Hrs Later	scrlc.pk003.g7
sdp4c	Soybean Developing Pod (10-12 mm)	sdp4c.pk003.h2
sfl1	Soybean Immature Flower	sfl1.pk131.g9
src3c	Soybean 8 Day Old Root Infected With Cyst Nematode	src3c.pk026.o11
wdk1c	Wheat Developing Kernel, 3 Days After Anthesis	wdk1c.pk008.g12
wdr1f	Wheat Developing Root (Full Length)	wdr1f.pk001.g9
wle1n	Wheat Leaf From 7 Day Old Etiolated Seedling*	wle1n.pk0109.h1

*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA

ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding auxin transport protein were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Auxin Transport Protein

The BLASTX search using the EST sequences from clones p0016.ctsag12r, p0119.cmtnl24r and wle1n.pk0109.h1, and the contig assembled from EST sequences from clones p0097.cqrai63r and p0094.csssh17r revealed similarity of the proteins encoded by the cDNAs to the auxin transport protein encoded by REH1 (Rice EIR1 Homolog) from rice (NCBI Gene Identifier No. 3377509). The BLAST results for each of these ESTs are shown in Table 3:

TABLE 3

BLAST Results for Clones Encoding Polypeptides Homologous to REH1 Protein

Clone	BLAST pLog Score 3377509
p0016.ctsag12r	10.5
Contig of: p0097.cqrai63r p0094.csssh17r	40.7
p0119.cmtnl24r	34.4
wle1n.pk0109.h1	52.0

The BLASTX search using the EST sequences from clones rsl1n.pk003.n3, src3c.pk026.o11 and wdk1c.pk008.g12 revealed similarity of the proteins encoded by the cDNAs to the auxin transport protein encoded by EIR1 from *Arabidopsis thaliana* (NCBI Gene Identifier No. 3377507). The BLAST results for each of these ESTs are shown in Table 4:

TABLE 4

BLAST Results for Clones Encoding Polypeptides Homologous to EIR1 Protein

Clone	BLAST pLog Score 3377507
rsl1n.pk003.n3	38.2
src3c.pk026.o11	39.2
wdk1c.pk008.g12	41.0

The BLASTX search using the EST sequences from clone sfl1.pk131.g9 revealed similarity of the protein encoded by the cDNA to the auxin transport protein encoded by PIN1 from *Arabidopsis thaliana* (NCBI Gene Identifier No. 4151319) with a pLog value of 30.2. The BLASTX search using the EST sequences from clone sdp4c.pk003.h2 revealed similarity of the protein encoded by the cDNA to a putative auxin transport protein encoded by a gene from *Arabidopsis thaliana* (NCBI Gene Identifier No. 3785972) with a pLog value of 37.7.

The sequence of a substantial portion of the cDNA insert from clone p0016.ctsag12r is shown in SEQ ID NO:5; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:6. The sequence of a contig assembled from a portion of the cDNA insert from clones p0097.cqrai63r and p0094.csssh17r is shown in SEQ ID NO:7; the deduced amino acid sequence of this contig is shown in SEQ ID NO:8. The sequence of a substantial portion of the cDNA insert from clone p0119.cmtnl24r is shown in SEQ ID NO:11; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:12. The sequence of a substantial portion of the cDNA insert from clone rsl1n.pk003.n3

is shown in SEQ ID NO:17; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:18. The sequence of a substantial portion of the cDNA insert from clone sdp4c.pk003.h2 is shown in SEQ ID NO:23; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:24. The sequence of a substantial portion of the cDNA insert from clone sfl1.pk131.g9 is shown in SEQ ID NO:27; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:28. The sequence of a substantial portion of the cDNA insert from clone src3c.pk026.o11 is shown in SEQ ID NO:31; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:32. The sequence of a substantial portion of the cDNA insert from clone wdk1c.pk008.g12 is shown in SEQ ID NO:35; the deduced amino acid sequence of this portion of the cDNA is shown in SEQ ID NO:36. The sequence of a substantial portion of the cDNA insert from wle1n.pk0109.h1 is shown in SEQ ID NO:41; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:42. BLAST scores and probabilities indicate that the instant nucleic acid fragments encode portions of auxin transport proteins.

The BLASTX search using the EST sequences from clones listed in Table 5 revealed similarity of the polypeptides encoded by the cDNAs to auxin transport proteins from rice (NCBI GenBank Identifier (GI) Nos. 3377509 and 7489524) and Arabidopsis (NCBI GenBank Identifier (GI) Nos. 5902405, 5817301, 4151319, 3377507, and 3785972). Shown in Table 5 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 5

BLAST Results for Sequences Encoding Polypeptides Homologous to Auxin Transport Protein

		BLAST Results	
Clone	Status	NCBI GenBank Identifier (GI) No.	pLog Score
ceb1.pk0082.a5	EST	3377509	79.10
Contig of: cr1.pk0022.a4 cr1n.pk0033.e3 csi1n.pk0045.a5 csi1n.pk0050.d5 p0005.cbmej72r p0041.crtba02r	Contig	3377509	91.70
p0094.csssh17r	FIS	3377509	>254.00
p0119.cmtnl24r (FIS)	CGS	7489524	180.00
cil1.pk001.b7	FIS	7489524	135.00
rr1.pk0019.c4	EST	5902405	33.30

Clone	Status	BLAST Results NCBI GenBank Identifier (GI) No.	pLog Score
rsl1n.pk003.n3	FIS	5817301	155.00
scr1c.pk003.g7	FIS	4151319	170.00
sdp4c.pk003.h2	FIS	5817301	>254.00
sfl1.pk131.g9(FIS)	CGS	4151319	>254.00
src3c.pk026.o11(FIS)	CGS	3377507	>254.00
wdk1c.pk008.g12(FIS)	CGS	3377507	>254.00
wdr1f.pk001.g9	EST	3785972	27.30
wle1n.pko109.hl	FIS	3377509	48.00

Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:14, 30, 34, and 38, the auxin transport protein EIR1 sequence from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 3377507; SEQ ID NO:43), and the auxin transport protein AtPIN1 sequence from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 4151319; SEQ ID NO:44). The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:14, 30, 34, and 38, the auxin transport protein EIR1 sequence from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 3377507; SEQ ID NO:43), and the auxin transport protein AtPIN1 from Arabidopsis thaliana (NCBI GenBank Identifier (GI) No. 4151319; SEQ ID NO:44).

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Auxin Transport Protein

SEQ ID NO.	Percent Identity to SEQ ID NO:43	SEQ ID NO:44
14	51.5	55.3
30	57.9	72.3
34	75.1	59.6
38	59.7	52.1

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments, BLAST scores and

probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode all or a substantial portion of an auxin transport protein.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptide in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptide, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the

tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* *8*:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

A seed-specific construct composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin construct includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire construct is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed construct.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase

gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed construct comprising the phaseolin 5' region, the fragment encoding the instant polypeptide and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μL of a 60 mg/mL 1 μm gold particle suspension is added (in order): 5 μL DNA (1 μg/μL), 20 μL spermidine (0.1 M), and 50 μL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μL 70% ethanol and resuspended in 40 μL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using

oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 μg/mL ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene encoding the instant polypeptide are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 μL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 7

Evaluating Compounds for Their Ability to Inhibit the Activity of Auxin Transport Proteins

The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant

polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("$(His)_6$"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a $(His)_6$ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β–mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the auxin transport proteins disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for auxin transport proteins are presented by Chen, R. et al., (1998) *Proc. Natl. Acad. Sci. USA 95*:15112-15117.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence encoding a polypeptide of at least 30 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6;

(b) a second nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40;

(c) a third nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12;

(d) a fourth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24;

(e) a fifth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32;

(f) a sixth nucleotide sequence encoding a polypeptide of at least 90 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42;

(g) a seventh nucleotide sequence encoding a polypeptide of at least 95 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46;

(h) an eighth nucleotide sequence encoding a polypeptide of at least 100 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20;

(i) a ninth nucleotide sequence encoding a polypeptide of at least 100 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2;

(j) a tenth nucleotide sequence encoding a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4;

(k) an eleventh nucleotide sequence encoding a polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38;

(l) a twelfth nucleotide sequence encoding a polypeptide of at least 350 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10;

(m) a thirteenth nucleotide sequence encoding a polypeptide of at least 400 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30;

(n) a fourteenth nucleotide sequence encoding a polypeptide of at least 500 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34;

(o) a fifteenth nucleotide sequence encoding a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14;

(p) a sixteenth nucleotide sequence encoding a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48; and

(q) a seventeenth nucleotide sequence comprising a complement of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o) or (p).

2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.

3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.

4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.

5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

6. A host cell comprising the chimeric gene of Claim 5.

7. A host cell comprising the isolated polynucleotide of Claim 1.

8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, and plant.

9. A virus comprising the isolated polynucleotide of Claim 1.

10. A polypeptide selected from the group consisting of:

(a) a polypeptide of at least 30 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6;

(b) a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40;

(c) a polypeptide of at least 50 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12;

(d) a polypeptide of at least 50 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24;

(e) a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32;

(f) a polypeptide of at least 90 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42;

(g) a polypeptide of at least 95 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46;

(h) a polypeptide of at least 100 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20;

(i) a polypeptide of at least 100 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2;

(j) a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4;

(k) a polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38;

(l) a polypeptide of at least 350 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10;

(m) a polypeptide of at least 400 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30;

(n) a polypeptide of at least 500 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34;

(o) a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; and

(p) a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48.

11. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a plant cell, the method comprising the steps of:

(a) constructing the isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from the isolated polynucleotide of Claim 1;

(b) introducing the isolated polynucleotide into the plant cell;

(c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the isolated polynucleotide.

12. The method of Claim 11 wherein the isolated polynucleotide consists of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.

13. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a plant cell, the method comprising the steps of:

(a) constructing the isolated polynucleotide of Claim 1;

(b) introducing the isolated polynucleotide into the plant cell;

(c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the polynucleotide.

14. A method of obtaining a nucleic acid fragment encoding a polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and a complement of such nucleotide sequences; and

(b) amplifying the nucleic acid sequence using the oligonucleotide primer.

15. A method of obtaining a nucleic acid fragment encoding a polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and a complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

(d) sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.

16. A method for evaluating at least one compound for its ability to inhibit the activity of a protein, the method comprising the steps of:

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding the polypeptide, operably linked to at least one suitable regulatory sequence;

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the auxin transport protein encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the auxin transport polypeptide expressed by the transformed host cell;

(d) treating the auxin transport polypeptide with a compound to be tested; and

(e) comparing the activity of the auxin transport polypeptide that has been treated with the test compound to the activity of an untreated auxin transport polypeptide,

thereby selecting compounds with potential for inhibitory activity.

17. A composition comprising the isolated polynucleotide of Claim 1.

18. A composition comprising the isolated polypeptide of Claim 10.

19. The isolated polynucleotide of Claim 1 comprising a nucleotide sequence having at least one of 30 contiguous nucleotides.

20. A method for positive selection of a transformed cell comprising:

(a) transforming a host cell with the chimeric gene of Claim 5; and

(b) growing the transformed host cell under conditions which allow expression of a polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

21. The method of Claim 20 wherein the host cell is a plant.

22. The method of Claim 21 wherein the plant cell is a monocot.

23. The method of Claim 21 wherein the plant cell is a dicot.

24. A method of modulating expression of a polypeptide for modulating root development in a plant, comprising the steps of:

(a) stably transforming a plant cell with an auxin transport protein polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation; and

(b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate root development in the plant.

FIGURE 1

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                   ***  * * *  * *******  ***** ** ** ************ ***********
SEQ ID NO:14       MITALDLYHVLTAVVPLYVAMTLAYGSVRWWRIFTPDQCSGINRFVALFAVPLLSFHFIS
SEQ ID NO:30       MITLTDFYHVMTAMVPLYVAMILAYGSVKWWKIFSPDQCSGINRFVALFAVPLLSFHFIA
SEQ ID NO:34       MITGKDIYDVFAAIVPLYVAMILAYGSVRWWKIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:38       MITGKDIYDVLAAVVPLYVAMFMAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:43       MITGKDMYDVLAAMVPLYVAMILAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
SEQ ID NO:44       MITAADFYHVMTAMVPLYVAMILAYGSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFIA
                   1                                                          60

                    * *  *   * *** ***   *  *     *      *          * * ******
SEQ ID NO:14       TNDPFAMNLRFLAADTLQKVAVLALLALASRGLSSPRALG---------LDWSITLFSLS
SEQ ID NO:30       SNNPYEMNLRFLAADTLQKIIILVLLAVW----SNITKRG--------CLEWAITLFSLS
SEQ ID NO:34       SNDPYAMNYHFIAADCLQKVVILGALFLWNT----FTKHG--------SLDWTITLFSLS
SEQ ID NO:38       TNDPYAMDYRFLAADSLQKLVILAALAVWHNVLSRYRCRGGTEAGEASSLDWTITLFSLA
SEQ ID NO:43       SNDPYAMNYHFLAADSLQKVVILAALFLWQA----FSRRG--------SLEWMITLFSLS
SEQ ID NO:44       ANNPYAMNLRFLAADSLQKVIVLSLLFLW----CKLSRNG--------SLDWTITLFSLS
                   61                                                        120

                   *************  ***  * * **** ****  ******** ** * *  *   ***
SEQ ID NO:14       TLPNTLVMGIPLLRGMYGASSAGTLMVQVVVLQCIIWYTLMLFLFEYRAARALVLDQFPD
SEQ ID NO:30       TLPNTLVMGIPLLKGMYGDFS-GSLMVQIVVLQCIIWYTLMLFLFEFRGARMLISEQFP-
SEQ ID NO:34       TLPNTLVMGIPLLKAMYGDFS-GSLMVQIVVLQSVIWYTLMLFMFEYRGAKLLITEQFP-
SEQ ID NO:38       TLPNTLVMGIPLLRAMYGDFS-GSLMVQIVVLQSVIWYTLMLFLFEYRGAKALISEQFPP
SEQ ID NO:43       TLPNTLVMGIPLLRAMYGDFS-GNLMVQIVVLQSIIWYTLMLFLFEFRGAKLLISEQFP-
SEQ ID NO:44       TLPNTLVMGIPLLKGMYGNFS-GDLMVQIVVLQCIIWYILMLFLFEYRGAKLLISEQFP-
                   121                                                       180
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FIGURE 1 CONTINUED

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                    ** *  ****  **  *   *                    *   *    *
SEQ ID NO:14    GAAASIVSFRVDSDVVSLARGDVELEAEPDGVAGAGAVSSRGGDAGRVRVTVRKSTSSRS
SEQ ID NO:30    DTAASIVSIHVDSDVMSLD-GRQPLETEAEI-----------KEDGKLHVTVRKSNASRS
SEQ ID NO:34    ETAGSITSFRVDSDVVSLN-GREPLQTDAEI-----------GEDGKLHVVVKRS-AASS
SEQ ID NO:38    DVGASIASFRVDSDVVSLN-GREALHADAEV-----------GRDGRVHVVIRRSASGST
SEQ ID NO:43    ETAGSITSFRVDSDVISLN-GREPLQTDAEI-----------GDDGKLHVVVRRSSAASS
SEQ ID NO:44    DTAGSIVSIHVDSDIMSLD-GRQPLETEAEI-----------KEDGKLHVTVRRSNASRS
               181                                                       240

                                    ** ***   **** * ** **** ****  **       *
SEQ ID NO:14    EAACSHSHSQ-------TMQPRVSNLSGVEIYSLQSSRNPTPRGSSFNHADFFNIVGAA-
SEQ ID NO:30    DI---FSRR---SQGLSSTTPRPSNLTNAEIYSLQSSRNPTPRGSSFNHTDFYSMMAAG-
SEQ ID NO:34    MIS-SFNKSHLTSM-----TPRASNLTGVEIYSVQSSREPTPRGSSFNQTDFYAMF-ASK
SEQ ID NO:38    TGGHGAGRSGIYRGASNAMTPRASNLTGVEIYSLQTSREPTPRQSSFNQSDFYSMFNGSK
SEQ ID NO:43    MIS-SFNKSHGGGLNSSMITPRASNLTGVEIYSVQSSREPTPRASSFNQTDFYAMFNASK
SEQ ID NO:44    DI---YSRR---SQGLSAT-PRPSNLTNAEIYSLQSSRNPTPRGSSFNHTDFYSMMASG-
               241                                                       300

                  * * *               *  *  * * * *     *      **    *
SEQ ID NO:14    ---AKGG--------GGAAGDE--------------------------------------
SEQ ID NO:30    -----G-RNSNF-----GASD-VYGLSASRGPTPRPSNYDEDGGKP--K-----------
SEQ ID NO:34    APSPKHGYTNSFQSNNGGIG-DVYSLQSSKGATPRTSNFEEEMLKMHKK--RGGRSMSGE
SEQ ID NO:38    LASPKG------QPPVAGGGG------------ARGQGLDEQVANK--------------
SEQ ID NO:43    APSPRHGYTNSYGGAGAGPGGDVYSLQSSKGVTPRTSNFDEEVMKTAKKAGRGGRSMSGE
SEQ ID NO:44    -----GGRNSNF-----GPGEAVFG---SKGPTPRPSNYEEDGGPA--KPTAAGTAAGAG
               301                                                       360
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FIGURE 1 CONTINUED

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                  *  *      * *  *                          *
SEQ ID NO:14  ----EKGACGGGGGGHSPQPQA-------------------------VAVPAKRKDLHM
SEQ ID NO:30  -FHYHA----AGGTGHYPAPNPGMFSPSNGSKSVAANANAKRPNGQAQLKPEDGNRDLHM
SEQ ID NO:34  LFN------GGLVSSNYPPPNP-MFSGSTSAAGGPKKKDSSGGG-----GAVAPNKELHM
SEQ ID NO:38  -FK------GGEAAAPYPAPNP-GMM-----MPAPRKKELGGSNSNS-------DKELHM
SEQ ID NO:43  LYN------NNSVPS-YPPPNP-MFTGSTSGASGVKKKESGGGGSGGGVGVGGQNKEMNM
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SEQ ID NO:34  FVWSSSASPVSEGNLRHAVNRAASTDFGTVDPSKAVPHETVASKAVHELIENMSPGRRGS
SEQ ID NO:38  FVWSSSASPVSEANLRNAVNHAASTDFAAAPPAAATPRDGATPRGVSGSVTPVMKKDASS
SEQ ID NO:43  FVWSSSASPVSEANAKNAMTRGSSTDVSTDPKVSIPPHDNLATKAMQNLIENMSPGRKGH
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SEQ ID NO:34  GEREPEMDEG----------AKIPASGSPYTCQKKVDMEDGNAN-KNQQMPPASVMTRLI
SEQ ID NO:38  GAVEVEIEDGMMKSPATGLGAKFPVSGSPYVAPRKKGADVPGLEEAAHPMPPASVMTRLI
SEQ ID NO:43  VEMDQDGNNG----------GK-----SPYMGKKGSDVEDGGPGPRKQQMPPASVMTRLI
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FIGURE 1 CONTINUED

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SEQ ID NO:38     LIMVWRKLIRNPNTYSSLIGLVWSLVSFRWNIQMPTIIKGSISILSDAGLGMAMFSLGLF
SEQ ID NO:43     LIMVWRKLIRNPNTYSSLFGLAWSLVSFKWNIKMPTIMSGSISILSDAGLGMAMFSLGLF
SEQ ID NO:44     LIMVWRKLIRNPNSYSSLFGITWSLISFKWNIEMPALIAKSISILSDAGLGMAMFSLGLF
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SEQ ID NO:34     MALQPKIIACGKSVAAFSMAVRFLTGPAVIAATSIGIGLRGVLLHVAIVQAALPQGIVPF
SEQ ID NO:38     MALQPKIISCGKSVATFAMAVRFLTGPAVIAATSIAVGLRGVLLHVAIVQAALPQGIVPF
SEQ ID NO:43     MALQPKIIACGKSVAGFAMAVRFLTGPAVIAATSIAIGIRGDLLHIAIVQAALPQGIVPF
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SEQ ID NO:34     VFAKEYNLHADILSTAVIFGMLIALPITILYYVLLGV
SEQ ID NO:38     VFAKEYNCHPQILSTAVIFGMLVALPITILYYVLLGI
SEQ ID NO:43     VFAKEYNVHPDILSTAVIFGMLVALPVTVLYYVLLGL
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35 40 45

Pro Ala Ile Ile Ala Arg Ser Ile Ser Ile Leu Ser Asp Ala Gly Leu
50 55 60

Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln Pro Arg
65 70 75 80

Ile Ile Ala Cys Gly Asn Lys Leu Ala Ala Ile Ala Met Gly Val Arg
85 90 95

Phe Val Ala Gly Pro Ala Val Met Ala Ala Ala Ser Ile Ala Val Gly
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Leu Arg Gly Val Leu Leu His Ile Ala Ile Val Gln Ala Ala Leu Pro
115 120 125

Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Gly Val His Pro
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            20                  25                  30

Met Val Trp Arg Gln Leu Ile Arg Asn Pro Asn Thr Tyr Ser Ser Leu
        35                  40                  45

Ile Gly Val Ile Trp Ser Leu Val Cys Phe Arg Trp Asn Phe Gln Met
    50                  55                  60

Pro Ala Ile Val Leu Gln Ser Ile Ser Ile Leu Ser Asp Ala Gly Leu
65                  70                  75                  80

Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln Pro Arg
                85                  90                  95

Ile Ile Ala Cys Gly Asn Lys Val Ala Thr Phe Ala Met Ala Val Arg
            100                 105                 110

Phe Leu Thr Gly Pro Ala Val Met Ala Ala Ala Ser Phe Ala Val Gly
        115                 120                 125

Leu Arg Gly Thr Leu Leu His Val Ala Ile Val Gln Ala Ala Leu Pro
    130                 135                 140

Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Val His Pro
145                 150                 155                 160

Asp Ile Leu Ser Thr Ala Val Ile Phe Gly Met Leu Ile Ala Leu Pro
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Ile Thr Leu Val Tyr Tyr Ile Leu Leu Gly Leu
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Ile Phe Ser Pro Glu Gln Cys Ser Gly Ile Asn Arg Phe Xaa Ala Leu
        35                  40                  45

Phe Xaa Val Pro Leu Leu Ser Phe His Phe Ile Ser Xaa Gln Gln Pro
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Ile Phe Ser Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asn Pro
    50                  55                  60

Tyr Thr Met Asn Leu Arg Phe Ile Ala Ala Asp Thr Leu Gln Lys Leu
65                  70                  75                  80

Met Val Leu Ala Met Leu Thr Ala Trp Ser His Leu Ser Arg Arg Gly
                85                  90                  95

Ser Leu Glu Trp Thr Ile Thr Leu Phe Ser Leu Ser Thr Leu Pro Asn
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Thr Leu Val Met Gly Ile Pro Leu Leu Lys Gly Met Tyr Gly Asp Phe
        115                 120                 125
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Ser Gly Ser Leu Met Val Gln Ile Val Val Leu Gln Cys Ile Ile Trp
    130                 135                 140

Tyr Thr Leu Met Leu Phe Met Phe Glu Tyr Arg Gly Ala Arg Met Leu
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Ile Thr Glu Gln Phe Pro Asp Asn Ala Gly Ala Ile Ala Ser Ile Val
                165                 170                 175

Val Asp Pro Asp Val Val Ser Leu Asp Gly Arg Arg Asp Ala Ile Glu
            180                 185                 190

Thr Glu Ala Glu Val Lys Glu Asp Gly Arg Ile His Val Thr Val Arg
        195                 200                 205

Arg Ser Asn Ala Ser Arg Ser Asp Ile Tyr Ser Arg Arg Ser Met Gly
    210                 215                 220

Phe Ser Ser Thr Thr Pro Arg Pro Ser Asn Leu Thr Asn Ala Glu Ile
225                 230                 235                 240

Tyr Ser Leu Gln Ser Ser Arg Asn Pro Thr Pro Arg Gly Ser Ser Phe
                245                 250                 255

Asn His Asn Asp Phe Tyr Ser Met Val Gly Arg Ser Ser Asn Phe Gly
            260                 265                 270

Ala Ala Asp Ala Phe Gly Ile Arg Thr Gly Ala Thr Pro Arg Pro Ser
        275                 280                 285

Asn Tyr Glu Asp Asp Ala Ser Lys Pro Lys Tyr Pro Leu Pro Val Val
    290                 295                 300

Asn Ala Thr Ser Gly Ala Gly Ala Ala His Tyr Pro Ala Pro Asn Pro
305                 310                 315                 320

Ala Val Ala Ala Ala Pro Lys Gly Ala Arg Lys Ala Ala Thr Asn Gly
                325                 330                 335

Gln Ala Lys Gly Glu Asp Leu His Met Phe Val Trp Ser Ser Ser Ala
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Ser Pro Val Ser Asp Val Phe Gly Gly Gly Ala Pro Asp Tyr Asn Glu
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Leu Tyr Val Ala Met Thr Leu Ala Xaa Gly Xaa Val Arg Trp Xaa Xaa
            20                  25                  30

Xaa Xaa Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asp Pro
    50                  55                  60

Phe Ala Met Asn Leu Arg Phe Leu Ala Val Asp Thr Leu Gln Lys Val
65                  70                  75                  80

Ala Val Leu Ala Leu Leu Ala Leu Xaa Ser Xaa Ala Ala Ser Ser Xaa
                85                  90                  95

Arg Xaa Arg Ser Gly Leu Asp Trp Ser Ile Lys Leu Xaa Xaa Leu Ser
            100                 105                 110

Thr Leu


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<213> Zea mays

<400> 14

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Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asp Pro
    50                  55                  60

Phe Ala Met Asn Leu Arg Phe Leu Ala Ala Asp Thr Leu Gln Lys Val
65                  70                  75                  80

Ala Val Leu Ala Leu Leu Ala Leu Ala Ser Arg Gly Leu Ser Ser Pro
                85                  90                  95

Arg Ala Leu Gly Leu Asp Trp Ser Ile Thr Leu Phe Ser Leu Ser Thr
            100                 105                 110

Leu Pro Asn Thr Leu Val Met Gly Ile Pro Leu Leu Arg Gly Met Tyr
        115                 120                 125

Gly Ala Ser Ser Ala Gly Thr Leu Met Val Gln Val Val Val Leu Gln
    130                 135                 140

Cys Ile Ile Trp Tyr Thr Leu Met Leu Phe Leu Phe Glu Tyr Arg Ala
145                 150                 155                 160
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Ala Arg Ala Leu Val Leu Asp Gln Phe Pro Asp Gly Ala Ala Ala Ser
                165                 170                 175

Ile Val Ser Phe Arg Val Asp Ser Asp Val Val Ser Leu Ala Arg Gly
            180                 185                 190

Asp Val Glu Leu Glu Ala Glu Pro Asp Gly Val Ala Gly Ala Gly Ala
        195                 200                 205

Val Ser Ser Arg Gly Gly Asp Ala Gly Arg Val Arg Val Thr Val Arg
    210                 215                 220

Lys Ser Thr Ser Ser Arg Ser Glu Ala Ala Cys Ser His Ser His Ser
225                 230                 235                 240

Gln Thr Met Gln Pro Arg Val Ser Asn Leu Ser Gly Val Glu Ile Tyr
                245                 250                 255

Ser Leu Gln Ser Ser Arg Asn Pro Thr Pro Arg Gly Ser Ser Phe Asn
            260                 265                 270

His Ala Asp Phe Phe Asn Ile Val Gly Ala Ala Ala Lys Gly Gly Gly
        275                 280                 285

Gly Ala Ala Gly Asp Glu Glu Lys Gly Ala Cys Gly Gly Gly Gly Gly
    290                 295                 300

Gly His Ser Pro Gln Pro Gln Ala Val Ala Val Pro Ala Lys Arg Lys
305                 310                 315                 320

Asp Leu His Met Leu Val Trp Ser Ser Ser Ala Ser Pro Val Ser Glu
                325                 330                 335

Arg Ala Ala Val His Val Phe Gly Ala Gly Gly Ala Asp His Ala Asp
            340                 345                 350

Val Leu Ala Lys Gly Ala Gln Ala Tyr Asp Glu Tyr Gly Arg Asp Asp
        355                 360                 365

Tyr Ser Ser Arg Thr Lys Asn Gly Ser Gly Gly Ala Asp Lys Gly Gly
    370                 375                 380

Pro Thr Leu Ser Lys Leu Gly Ser Asn Ser Thr Ala Gln Leu Tyr Pro
385                 390                 395                 400

Lys Asp Asp Gly Glu Gly Arg Ala Ala Ala Val Ala Met Pro Pro Ala
                405                 410                 415

Ser Val Met Thr Arg Leu Ile Leu Ile Met Val Trp Arg Lys Leu Ile
            420                 425                 430

Arg Asn Pro Asn Thr Tyr Ser Ser Leu Ile Gly Val Val Trp Ser Leu
        435                 440                 445

Val Ser Tyr Arg Trp Gly Ile Glu Met Pro Ala Ile Ile Ala Arg Ser
    450                 455                 460

Ile Ser Ile Leu Ser Asp Ala Gly Leu Gly Met Ala Met Phe Ser Leu
465                 470                 475                 480
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Gly Leu Phe Met Ala Leu Gln Pro Arg Ile Ile Ala Cys Gly Asn Lys
                485                 490                 495

Leu Ala Ala Ile Ala Met Gly Val Arg Phe Val Ala Gly Pro Ala Val
            500                 505                 510

Met Ala Ala Ala Ser Ile Ala Val Gly Leu Arg Gly Val Leu Leu His
        515                 520                 525

Ile Ala Ile Val Gln Ala Ala Leu Pro Gln Gly Ile Val Pro Phe Val
    530                 535                 540

Phe Ala Lys Glu Tyr Gly Val His Pro Asp Ile Leu Ser Thr Ala Tyr
545                 550                 555                 560

Gly Pro Ile Thr Ser His Gly Phe Ile Thr Cys His Ser
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tcaatcgatt nttcttcgtt ttncaaagga gtatggctta ttncgatgac tcagnacggc 480
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<212> PRT
<213> Oryza sativa

<220>
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Val Gly Leu Lys Leu Ala Arg Asn Pro Asn Val Tyr Ala Ser Val Leu
1 5 10 15

Gly Val Val Trp Ala Cys Ile Ala Tyr Arg Trp His Leu Ser Leu Pro
20 25 30

Gly Ile Val Thr Gly Ser Leu Gln Val Met Ser Arg Thr Gly Thr Gly
35 40 45

Met Ser Met Phe Ser Met Gly Leu Phe Met Gly Gln Gln Glu Arg Val
50 55 60

Ile Ala Cys Gly Ala Gly Leu Thr Ala Leu Gly Met Ala Leu Arg Phe
65 70 75 80

Val Ala Gly Pro Leu Ala Thr Leu Val Gly Ala Ala Ala Leu Gly Leu
85 90 95

Arg Gly Asp Val Leu His Leu Ala Ile Ile Gln Xaa Xaa Leu
100 105 110

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<212> DNA
<213> Oryza sativa

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<210> 18
<211> 74
<212> PRT
<213> Oryza sativa

<400> 18
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1 5 10 15

Leu Tyr Val Ala Met Phe Leu Ala Tyr Gly Ser Val Arg Trp Trp Gly
20 25 30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Ile
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asp Pro
50 55 60

Tyr Ala Met Asn Leu Arg Phe Leu Ala Ala
65 70

<210> 19
<211> 2162
<212> DNA
<213> Oryza sativa

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<212>  PRT
<213>  Oryza sativa

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  1               5                  10                  15

Leu Tyr Val Ala Met Phe Leu Ala Tyr Gly Ser Val Arg Trp Trp Gly
             20                  25                  30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Ile
         35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asp Pro
     50                  55                  60

Tyr Ala Met Asn Leu Arg Phe Leu Ala Ala Asp Thr Leu Gln Lys Leu
 65                  70                  75                  80

Leu Val Leu Ala Gly Leu Ala Ala Trp Ser Arg Leu Pro Ser Arg Thr
                 85                  90                  95

Gly Ala Pro Arg Leu Asp Trp Ser Ile Thr Leu Phe Ser Leu Ser Thr
            100                 105                 110

Leu Pro Asn Thr Leu Val Met Gly Ile Pro Leu Leu Ile Ala Met Tyr
        115                 120                 125
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Gly Pro Tyr Ser Gly Ser Leu Met Val Gln Ile Val Val Leu Gln Cys
130 135 140

Ile Ile Trp Tyr Thr Leu Met Leu Phe Leu Phe Glu Phe Arg Ala Ala
145 150 155 160

Arg Met Leu Ile Ala Asp Gln Phe Pro Asp Thr Ala Ala Ser Ile Val
165 170 175

Ser Leu His Val Asp Pro Asp Val Val Ser Leu Glu Gly Gly His Ala
180 185 190

Glu Thr Glu Ala Glu Val Ala Ala Asp Gly Arg Leu His Val Thr Val
195 200 205

Arg Arg Ser Ser Val Ser Arg Arg Ser Leu Leu Val Thr Pro Arg Pro
210 215 220

Ser Asn Leu Thr Gly Ala Glu Ile Tyr Ser Leu Ser Ser Ser Arg Asn
225 230 235 240

Pro Thr Pro Arg Gly Ser Asn Phe Asn His Ala Asp Phe Phe Ala Met
245 250 255

Val Gly Gly Gly Pro Pro Pro Pro Thr Pro Ala Ala Val Arg Gly Ser
260 265 270

Ser Phe Gly Ala Ser Glu Leu Tyr Ser Leu Gln Ser Ser Arg Gly Pro
275 280 285

Thr Pro Arg Gln Ser Asn Phe Asp Glu His Ser Ala Arg Pro Pro Lys
290 295 300

Pro Pro Ala Thr Thr Thr Gly Ala Leu Asn His Asp Ala Lys Glu Leu
305 310 315 320

His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val Ser Glu Val Ser
325 330 335

Gly Leu Pro Val Phe Ser Gly Gly Gly Gly Gly Gly Ala Leu Asp Val
340 345 350

Gly Ala Lys Glu Ile His Met Val Ile Pro Ala Asp Leu Pro Gln Asn
355 360 365

Asn Gly Ser Gly Lys Glu His Glu Glu Tyr Gly Ala Val Ala Leu Gly
370 375 380

Gly Gly Gly Gly Gly Glu Asn Phe Ser Phe Gly Gly Gly Lys Thr Val
385 390 395 400

Asp Gly Ala Glu Ala Val Asp Glu Glu Ala Ala Leu Pro Asp Gly Leu
405 410 415

Thr Lys Met Gly Ser Ser Ser Thr Ala Glu Leu His Pro Lys Val Val
420 425 430

Asp Val Asp Gly Pro Asn Ala Gly Gly Gly Ala Ala Gly Ala Gly Gln
435 440 445

Tyr Gln Met Pro Pro Ala Ser Val Met Thr Arg Leu Ile Leu Ile Met
450 455 460

Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser Ser Leu Leu
465 470 475 480

Gly Leu Ala Trp Ser Leu Val Ala Phe Arg Leu Phe Met Ala Leu Gln
485 490 495

Pro Ser Ile Ile Ala Cys Gly Lys Ser Ala Ala Val Val Ser Met Ala
500 505 510

Val Arg Phe Leu Ala Gly Pro Ala Val Met Ala Ala Ala Ser Ile Ala
515 520 525

Ile Gly Leu Arg Gly Thr Leu Leu His Val Ala Ile Val Gln Ala Ala
530 535 540

Leu Pro Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Val
545 550 555 560

His Pro Ala Ile Leu Ser Thr Ala Val Ile Phe Gly Met Leu Ile Ala
565 570 575

Leu Pro Ile Thr Leu Leu Tyr Tyr Ile Leu Leu Gly Leu
580 585

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<211> 1618
<212> DNA
<213> Glycine max

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<212> PRT
<213> Glycine max

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Thr Asp Ala Gln Ile Lys Glu Asp Gly Lys Leu His Val Thr Val Arg
35 40 45

Lys Ser Asn Ala Ser Arg Ser Asp Ile Phe Ser Arg Arg Ser Gln Gly
50 55 60

Phe Ser Ser Thr Thr Pro Arg Pro Ser Asn Leu Thr Asn Ala Glu Ile
65 70 75 80

Tyr Ser Leu Gln Ser Ser Arg Asn Pro Thr Pro Arg Gly Ser Ser Phe
85 90 95

Asn His Thr Asp Phe Tyr Ser Met Met Ala Ala Gly Arg Asn Ser Asn
100 105 110

Phe Gly Ala Asn Asp Val Tyr Gly Leu Ser Ala Ser Arg Gly Pro Thr
115 120 125

Pro Arg Pro Ser Asn Tyr Asp Glu Asp Ala Ser Asn Asn Asn Asn Gly
130 135 140

Lys Pro Arg Tyr His Tyr Pro Ala Ala Gly Thr Gly Thr Gly Thr Gly
145 150 155 160

Thr Gly Thr Gly Thr Gly Thr Gly His Tyr Pro Ala Pro Asn Pro Gly
165 170 175

Met Phe Ser Pro Thr Ala Ser Lys Asn Val Ala Lys Lys Pro Asp Asp
180 185 190

Pro Asn Lys Asp Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro
195 200 205

Val Ser Asp Val Phe Gly Gly Gly His Glu Tyr Asp His Lys Glu Leu
210 215 220

Lys Leu Thr Val Ser Pro Gly Lys Val Glu Gly Asn Ile Asn Arg Asp
225 230 235 240

Thr Gln Glu Glu Tyr Gln Pro Glu Lys Asp Glu Phe Ser Phe Gly Asn
245 250 255

Arg Gly Ile Glu Asp Glu His Glu Gly Glu Lys Val Gly Asn Gly Asn
260 265 270

Pro Lys Thr Met Pro Pro Ala Ser Val Met Thr Arg Leu Ile Leu Ile
275 280 285

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Met Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser Ser Leu
    290                 295                 300

Ile Gly Leu Thr Trp Ser Leu Ile Ser Phe Arg Trp Asn Val Lys Met
305                 310                 315                 320

Pro Ala Ile Ile Ala Lys Ser Ile Ser Ile Leu Ser Asp Ala Gly Leu
                325                 330                 335

Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln Pro Arg
            340                 345                 350

Ile Ile Ala Cys Gly Asn Ser Thr Ala Ala Phe Ser Met Ala Val Arg
        355                 360                 365

Phe Leu Thr Gly Pro Ala Val Met Ala Ala Ala Ser Ile Ala Val Gly
    370                 375                 380

Leu Lys Gly Val Leu Leu His Val Ala Ile Val Gln Ala Ala Leu Pro
385                 390                 395                 400

Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Val His Pro
                405                 410                 415

Asp Ile Leu Ser Thr Gly Val Ile Phe Gly Met Leu Ile Ala Leu Pro
            420                 425                 430

Ile Thr Leu Val Tyr Tyr Ile Leu Leu Gly Leu
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<210> 23
<211> 531
<212> DNA
<213> Glycine max

<220>
<221> unsure
<222> (530)

<400> 23

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<211> 90
<212> PRT
<213> Glycine max

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<221> UNSURE
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<220>
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Arg Trp Trp Lys
20 25 30

Xaa Ile Phe Ser Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala
35 40 45

Ile Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asn
50 55 60

Pro Tyr Ala Met Asn Phe Arg Phe Ile Arg Arg Arg Thr Xaa Thr Ser
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Lys Lys Ile Ile Met Leu Phe Ala Leu Ala
85 90

<210> 25
<211> 2101
<212> DNA
<213> Glycine max

<400> 25
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aaccaaaatt ttccaattag cactagtagt acagtacaaa aaactagaag agcaaccaaa 180
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<210>  26
<211>  540
<212>  PRT
<213>  Glycine max

<400>  26
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Arg Trp Trp Lys
            20                  25                  30

Ile Phe Ser Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Ile
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asn Pro
    50                  55                  60

Tyr Ala Met Asn Phe Arg Phe Ile Ala Ala Asp Thr Leu Gln Lys Ile
65                  70                  75                  80

Ile Met Leu Phe Ala Leu Ala Ile Trp Thr Asn Leu Thr Lys Thr Gly
                85                  90                  95

Ser Leu Glu Trp Met Ile Thr Ile Phe Ser Leu Ser Thr Leu Pro Asn
            100                 105                 110

Thr Leu Val Met Gly Ile Pro Leu Leu Ile Ala Met Tyr Gly Asp Tyr
        115                 120                 125

Ser Gly Ser Leu Met Val Gln Val Val Val Leu Gln Cys Ile Ile Trp
    130                 135                 140

Tyr Thr Leu Leu Leu Phe Leu Phe Glu Tyr Arg Ala Ala Lys Ile Leu
145                 150                 155                 160

Ile Met Glu Gln Phe Pro Glu Thr Ala Ala Ser Ile Val Ser Phe Lys
                165                 170                 175

Val Asp Ser Asp Val Val Ser Leu Asp Gly Arg Asp Phe Leu Glu Thr
            180                 185                 190

Asp Ala Glu Val Gly Asp Asp Gly Lys Leu His Val Thr Val Arg Lys
        195                 200                 205

Ser Asn Ala Ser Arg Arg Ser Phe Met Met Thr Pro Arg Pro Ser Asn
    210                 215                 220

Leu Thr Gly Ala Glu Ile Tyr Ser Leu Ser Ser Ser Arg Asn Pro Thr
225                 230                 235                 240

Pro Arg Gly Ser Asn Phe Asn His Ala Asp Phe Phe Ser Met Met Gly
                245                 250                 255
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Tyr Gln Pro Arg His Ser Asn Phe Thr Ala Asn Asp Leu Phe Ser Ser
            260                 265                 270

Arg Gly Pro Thr Pro Arg Pro Ser Asn Phe Glu Glu Pro Ser Met Pro
        275                 280                 285

Gln Ala Val Thr Val Ala Ser Pro Arg Phe Gly Phe Tyr Pro Ser Gln
    290                 295                 300

Thr Val Pro Ala Ser Tyr Pro Pro Pro Asn Pro Asp Phe Ser Ser Ala
305                 310                 315                 320

Thr Lys Asn Leu Lys Asn Gln Ser Gln Asn Gln Asn Pro Asn Gln Ser
                325                 330                 335

Gln Ser Gln Asn Ser Gln Ala Pro Ala Lys Gly Ala His Asp Ala Lys
            340                 345                 350

Glu Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Met Ser Glu
        355                 360                 365

Asn Ala Gly Leu Asn Val Phe Ser Ser Thr Asp Leu Gly Thr Ser Glu
    370                 375                 380

Gln Pro Asp Gln Gly Ala Lys Glu Ile Arg Met Leu Val Ala Asp Asn
385                 390                 395                 400

Asn Ala His Leu Arg Asn Gly Glu Ala Asn Asn Lys Gly Gly Leu Glu
                405                 410                 415

Ala Val Leu Gly Val Glu Asp Phe Lys Phe Leu Val Asn Gly Glu Glu
            420                 425                 430

Gln Val Gly Glu Glu Lys Glu Gly Leu Asn Asn Gly Leu Asn Lys Leu
        435                 440                 445

Gly Ser Ser Ser Thr Val Glu Leu Gln Pro Lys Ala Thr Val Ala Gly
    450                 455                 460

Glu Ala Ser Ala Gly Lys His Met Pro Pro Ala Asn Val Met Thr Arg
465                 470                 475                 480

Leu Ile Leu Ile Met Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Thr
                485                 490                 495

Tyr Ser Ser Leu Ile Gly Val Val Trp Ser Leu Val Ala Phe Arg Trp
            500                 505                 510

His Val His Met Pro Lys Ile Ile Glu Lys Ser Ile Ser Ile Leu Ser
        515                 520                 525

Asp Ala Gly Leu Gly Met Ala Met Phe Ser Leu Gly
    530                 535                 540
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<210> 27
<211> 525
<212> DNA
<213> Glycine max

<400> 27
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caaaaccaca tgctcttcca catccctata taaaatcttt tcaatcttca taatcatcat 180
catcaccacc aactccaact caaactctcc aaaacctgcc acttcaacct tcctatatat 240
tccttccctc actctcttct gcttctatca tctttctgag aggcttgttg acacacaaaa 300
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<210> 28
<211> 64
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (38)

<400> 28
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Lys Trp Trp Lys
20 25 30

Ile Phe Ser Pro Asp Xaa Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ala Ser Asn Asn Pro
50 55 60

<210> 29
<211> 2549
<212> DNA
<213> Glycine max

<400> 29
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actgccccaa aaccacatgc tcttccacat ccctatataa aatcttttca atcttcataa 180
tcatcatcat caccaccaac tccaactcaa actctccaaa acctgccact tcaaccttcc 240
tatatattcc ttccctcact ctcttctgct tctatcatct ttctgagagg cttgttgaca 300
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<210> 30
<211> 605
<212> PRT
<213> Glycine max

<400> 30
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Lys Trp Trp Lys
20 25 30

Ile Phe Ser Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ala Ser Asn Asn Pro
50 55 60

Tyr Glu Met Asn Leu Arg Phe Leu Ala Ala Asp Thr Leu Gln Lys Ile
65 70 75 80

Ile Ile Leu Val Leu Leu Ala Val Trp Ser Asn Ile Thr Lys Arg Gly
85 90 95

Cys Leu Glu Trp Ala Ile Thr Leu Phe Ser Leu Ser Thr Leu Pro Asn
100 105 110

Thr Leu Val Met Gly Ile Pro Leu Leu Lys Gly Met Tyr Gly Asp Phe
115 120 125

Ser Gly Ser Leu Met Val Gln Ile Val Val Leu Gln Cys Ile Ile Trp
130 135 140

Tyr Thr Leu Met Leu Phe Leu Phe Glu Phe Arg Gly Ala Arg Met Leu
145 150 155 160

Ile Ser Glu Gln Phe Pro Asp Thr Ala Ala Ser Ile Val Ser Ile His
165 170 175

Val Asp Ser Asp Val Met Ser Leu Asp Gly Arg Gln Pro Leu Glu Thr
180 185 190

Glu Ala Glu Ile Lys Glu Asp Gly Lys Leu His Val Thr Val Arg Lys
195 200 205

Ser Asn Ala Ser Arg Ser Asp Ile Phe Ser Arg Arg Ser Gln Gly Leu
210 215 220

Ser Ser Thr Thr Pro Arg Pro Ser Asn Leu Thr Asn Ala Glu Ile Tyr
225 230 235 240

Ser Leu Gln Ser Ser Arg Asn Pro Thr Pro Arg Gly Ser Ser Phe Asn
245 250 255

His Thr Asp Phe Tyr Ser Met Met Ala Ala Gly Gly Arg Asn Ser Asn
260 265 270

Phe Gly Ala Ser Asp Val Tyr Gly Leu Ser Ala Ser Arg Gly Pro Thr
275 280 285

Pro Arg Pro Ser Asn Tyr Asp Glu Asp Gly Gly Lys Pro Lys Phe His
290 295 300

Tyr His Ala Ala Gly Gly Thr Gly His Tyr Pro Ala Pro Asn Pro Gly
305 310 315 320

Met Phe Ser Pro Ser Asn Gly Ser Lys Ser Val Ala Ala Asn Ala Asn
325 330 335

Ala Lys Arg Pro Asn Gly Gln Ala Gln Leu Lys Pro Glu Asp Gly Asn
340 345 350

Arg Asp Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val Ser
355 360 365

Asp Val Phe Gly Ala His Glu Tyr Gly Gly Gly His Asp Gln Lys Glu
370 375 380

Val Lys Leu Asn Val Ser Pro Gly Lys Val Glu Asn Asn His Arg Asp
385 390 395 400

Thr Gln Glu Asp Tyr Leu Glu Lys Asp Glu Phe Ser Phe Gly Asn Arg
405 410 415

Glu Met Asp Arg Glu Met Asn Gln Leu Glu Gly Glu Lys Val Gly Asp
420 425 430

Gly Lys Pro Lys Thr Met Pro Pro Ala Ser Val Met Thr Arg Leu Ile
435 440 445

Leu Ile Met Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser
450 455 460

Ser Leu Ile Gly Leu Thr Trp Ser Leu Val Ser Phe Lys Trp Asn Val
465 470 475 480

Glu Met Pro Ala Ile Ile Ala Lys Ser Ile Ser Ile Leu Ser Asp Ala
485 490 495

Gly Leu Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln
500 505 510

Pro Arg Val Ile Ala Cys Gly Asn Ser Thr Ala Ala Phe Ala Met Ala
515 520 525

Val Arg Phe Leu Thr Gly Pro Ala Val Met Ala Ala Ala Ser Ile Ala
530 535 540

Val Gly Leu Lys Gly Val Leu Leu His Val Ala Ile Val Gln Ala Ala
545 550 555 560

Leu Pro Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Val
565 570 575

His Pro Asp Ile Leu Ser Thr Ala Val Ile Phe Gly Met Leu Ile Ala
580 585 590

Leu Pro Ile Thr Leu Val Tyr Tyr Ile Leu Leu Gly Leu
595 600 605

<210> 31
<211> 419
<212> DNA
<213> Glycine max

<220>
<221> unsure
<222> (237)

<220>
<221> unsure
<222> (250)

<220>
<221> unsure
<222> (347)

<400> 31
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gtgttcgcag ttccacttct ttctttccac ttcatctcct ccaatgnccc ttatgctatg 360
aactaccact tcatagcagc tgattgtctt caaaaagttg tcattttggg gggctcccc 419

<210> 32
<211> 84
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (25)

<220>
<221> UNSURE
<222> (32)

<220>
<221> UNSURE
<222> (64)

<400> 32
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Leu Tyr Val Ala Met Ile Leu Ser Xaa Tyr Gly Ser Val Arg Trp Xaa
20 25 30

Lys Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala
35 40 45

Val Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Ser Asn Xaa
50 55 60

Pro Tyr Ala Met Asn Tyr His Phe Ile Ala Ala Asp Cys Leu Gln Lys
65 70 75 80

Val Val Ile Leu

<210> 33
<211> 2324
<212> DNA
<213> Glycine max

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cccataacca tactctacta cgtgctgctt ggagtttaat ttgtcttggg agacaaaagc 2100
aatagaaaaa gaagtatatg ttgctataac tgtacgtact atgtaaaccc aatgtcacgc 2160
tcaagcgggg tggatgaagg gaaatgtaga agatattgga ttttagatgt tagagggaaa 2220
gagaaattat atatagtata cggtagaatg ctatatatat taattattta tgattcatat 2280
gaaaattttg gtttgattcg ttccacaaaa aaaaaaaaaa aaaa 2324

<210> 34
<211> 637
<212> PRT
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<400> 34
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Arg Trp Trp Lys
20 25 30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Val
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Ser Asn Asp Pro
50 55 60

Tyr Ala Met Asn Tyr His Phe Ile Ala Ala Asp Cys Leu Gln Lys Val
65 70 75 80

Val Ile Leu Gly Ala Leu Phe Leu Trp Asn Thr Phe Thr Lys His Gly
85 90 95

Ser Leu Asp Trp Thr Ile Thr Leu Phe Ser Leu Ser Thr Leu Pro Asn
100 105 110

Thr Leu Val Met Gly Ile Pro Leu Leu Lys Ala Met Tyr Gly Asp Phe
115 120 125

Ser Gly Ser Leu Met Val Gln Ile Val Val Leu Gln Ser Val Ile Trp
130 135 140

Tyr Thr Leu Met Leu Phe Met Phe Glu Tyr Arg Gly Ala Lys Leu Leu
145 150 155 160

Ile Thr Glu Gln Phe Pro Glu Thr Ala Gly Ser Ile Thr Ser Phe Arg
165 170 175

Val Asp Ser Asp Val Val Ser Leu Asn Gly Arg Glu Pro Leu Gln Thr
180 185 190

Asp Ala Glu Ile Gly Glu Asp Gly Lys Leu His Val Val Val Lys Arg
195 200 205

Ser Ala Ala Ser Ser Met Ile Ser Ser Phe Asn Lys Ser His Leu Thr
210 215 220

Ser Met Thr Pro Arg Ala Ser Asn Leu Thr Gly Val Glu Ile Tyr Ser
225 230 235 240

Val Gln Ser Ser Arg Glu Pro Thr Pro Arg Gly Ser Ser Phe Asn Gln
245 250 255

Thr Asp Phe Tyr Ala Met Phe Ala Ser Lys Ala Pro Ser Pro Lys His
260 265 270

Gly Tyr Thr Asn Ser Phe Gln Ser Asn Asn Gly Gly Ile Gly Asp Val
275 280 285

Tyr Ser Leu Gln Ser Ser Lys Gly Ala Thr Pro Arg Thr Ser Asn Phe
290 295 300

Glu Glu Glu Met Leu Lys Met His Lys Lys Arg Gly Gly Arg Ser Met
305 310 315 320

Ser Gly Glu Leu Phe Asn Gly Gly Leu Val Ser Ser Asn Tyr Pro Pro
325 330 335

Pro Asn Pro Met Phe Ser Gly Ser Thr Ser Ala Ala Gly Gly Pro Lys
340 345 350

Lys Lys Asp Ser Ser Gly Gly Gly Gly Ala Val Ala Pro Asn Lys Glu
355 360 365

Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val Ser Glu Gly
370 375 380

Asn Leu Arg His Ala Val Asn Arg Ala Ala Ser Thr Asp Phe Gly Thr
385 390 395 400

Val Asp Pro Ser Lys Ala Val Pro His Glu Thr Val Ala Ser Lys Ala
405 410 415

Val His Glu Leu Ile Glu Asn Met Ser Pro Gly Arg Arg Gly Ser Gly
420 425 430

Glu Arg Glu Pro Glu Met Asp Glu Gly Ala Lys Ile Pro Ala Ser Gly
435 440 445

Ser Pro Tyr Thr Cys Gln Lys Lys Val Asp Met Glu Asp Gly Asn Ala
450 455 460

Asn Lys Asn Gln Gln Met Pro Pro Ala Ser Val Met Thr Arg Leu Ile
465 470 475 480

Leu Ile Met Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser
485 490 495

Ser Leu Leu Gly Leu Thr Trp Ser Leu Ile Ser Phe Arg Trp His Ile
500 505 510

Glu Met Pro Thr Ile Val Lys Gly Ser Ile Ser Ile Leu Ser Asp Ala
515 520 525

Gly Leu Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln
530 535 540

Pro Lys Ile Ile Ala Cys Gly Lys Ser Val Ala Ala Phe Ser Met Ala
545 550 555 560

Val Arg Phe Leu Thr Gly Pro Ala Val Ile Ala Ala Thr Ser Ile Gly
565 570 575

Ile Gly Leu Arg Gly Val Leu Leu His Val Ala Ile Val Gln Ala Ala
580 585 590

Leu Pro Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Leu
595 600 605

His Ala Asp Ile Leu Ser Thr Ala Val Ile Phe Gly Met Leu Ile Ala
610 615 620

Leu Pro Ile Thr Ile Leu Tyr Tyr Val Leu Leu Gly Val
625 630 635

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gncatgttca tggcgtacgg gtcggtgcgg tggtggggca tcttcacgcc ggaccantgc 180
tcgggcatca aacgcttcgt ngccgtcttc gcggtggcgc tcctctcctt ccacttcatc 240
tccaccaacg aaccctacgc catggactaa cgcttcctgg gcgccgactc gctgcanaan 300
ntcgttatcc tcgccgncct cgccgtgtgg ganaangtgc tctcccncca acggtgcccn 360

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gggggganaga aggcggcgaa ggctcctcnc tgggctggga caacanactc ttctccttgg 420
ggaaagtgcc aaaanactgg ngaaggggaa tccccctgct gggcgcaagt atg        473

<210>  36
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<212>  PRT
<213>  Triticum aestivum

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<220>
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Met Ile Thr Gly Lys Asp Ile Tyr His Xaa Leu Xaa Xaa Val Val Pro
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Leu Tyr Val Xaa Met Phe Met Ala Tyr Gly Ser Val Arg Trp Trp Gly
            20                  25                  30

Ile Phe Thr Pro Asp Xaa Cys Ser Gly Ile Lys Arg Phe Val Ala Val
        35                  40                  45

Phe Ala Val Ala Leu Leu Ser Phe His Phe Ile Ser Thr Asn Glu Pro
    50                  55                  60

Tyr Ala Met Asp Xaa Arg Phe Leu Gly Ala Asp Ser Leu Xaa Xaa Xaa
65                  70                  75                  80

Val Ile Leu Ala Xaa Leu Ala Val Trp
                85

<210>  37
<211>  2293
<212>  DNA
<213>  Triticum aestivum
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<400> 37

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gctgtacgtg gccatgttca tggcgtacgg gtcggtgcgg tggtggggca tcttcacgcc  180
ggaccagtgc tcgggcatca accgcttcgt cgccgtcttc gcggtgccgc tcctctcctt  240
ccacttcatc tccaccaacg acccctacgc catggactac cgcttcctgg ccgccgactc  300
gctgcagaag ctcgtcatcc tcgccgccct cgccgtgtgg cacaacgtgc tctcccgcta  360
ccggtgccgc ggcggcacgg aggccggcga ggcctcgtcg ctggactgga ccatcacgct  420
cttctccctg gcgacgctgc ccaacacgct ggtgatgggc atcccgctgc tgcgcgccat  480
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gtacacgctc atgctcttcc tcttcgagta ccgcggcgcc aaggcgctca tctccgagca  600
gttcccgccc gacgtcggcg ccagcatcgc ctccttccgc gtcgactccg acgtcgtctc  660
gctcaacggg cgcgaggcgc tgcacgccga cgccgaggtc ggccgcgacg gccgcgtcca  720
cgtcgtcatc cgccggtccg cgtcggggtc caccacgggc ggccacggcg ccgggcgctc  780
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ggagatctac tcgctgcaga cgtcgcggga gcccacgccg aggcagtcca gcttcaacca  900
gtccgacttc tactccatgt tcaacgggag caagctggct agtcccaagg gccagccccc  960
cgtcgccgga ggtggtggtg cgcgcgggca ggggctcgac gagcaggtgg ccaacaagtt 1020
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gccacggaag aaggagcttg gggttccaa ctcaaactcg aacaaggagc tgcacatgtt 1140
cgtgtggagc tccagcgcgt cgcccgtgtc ggaggccaac ctccgcaacg ccgtcaacca 1200
cgccgcgtcc accgacttcg ccgccgcacc gccggcggca gccacgccac gagacggcgc 1260
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cgcggtggag gtggagatcg aggacggcat gatgaagagc ccggcgacgg ggctgggcgc 1380
caagttcccg gtgtcggggt ccccctacgt ggccccgcgg aagaagggcg ccgacgtgcc 1440
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tcttgaagaa ccaaggctgc aaatcttcgg gtagggagaa gtagaattct agagagaaaa 2040
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aaggataggg agaactaagt aggaccctag acaggaattc aaaggacaga taaagatatc 2160
cttggttcca ttttttaat tttttatatt atttttacta ctgttttaga tccaaagtaa 2220
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aaaaaaaaaa aaa                                                    2293
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<210> 38
<211> 632
<212> PRT
<213> Triticum aestivum

<400> 38

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Leu Tyr Val Ala Met Phe Met Ala Tyr Gly Ser Val Arg Trp Trp Gly
            20                  25                  30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Val
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asp Pro
    50                  55                  60
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Tyr Ala Met Asp Tyr Arg Phe Leu Ala Ala Asp Ser Leu Gln Lys Leu
 65                 70                  75                  80

Val Ile Leu Ala Ala Leu Ala Val Trp His Asn Val Leu Ser Arg Tyr
                85                  90                  95

Arg Cys Arg Gly Gly Thr Glu Ala Gly Glu Ala Ser Ser Leu Asp Trp
            100                 105                 110

Thr Ile Thr Leu Phe Ser Leu Ala Thr Leu Pro Asn Thr Leu Val Met
        115                 120                 125

Gly Ile Pro Leu Leu Arg Ala Met Tyr Gly Asp Phe Ser Gly Ser Leu
    130                 135                 140

Met Val Gln Ile Val Val Leu Gln Ser Val Ile Trp Tyr Thr Leu Met
145                 150                 155                 160

Leu Phe Leu Phe Glu Tyr Arg Gly Ala Lys Ala Leu Ile Ser Glu Gln
                165                 170                 175

Phe Pro Pro Asp Val Gly Ala Ser Ile Ala Ser Phe Arg Val Asp Ser
            180                 185                 190

Asp Val Val Ser Leu Asn Gly Arg Glu Ala Leu His Ala Asp Ala Glu
        195                 200                 205

Val Gly Arg Asp Gly Arg Val His Val Val Ile Arg Arg Ser Ala Ser
    210                 215                 220

Gly Ser Thr Thr Gly Gly His Gly Ala Gly Arg Ser Gly Ile Tyr Arg
225                 230                 235                 240

Gly Ala Ser Asn Ala Met Thr Pro Arg Ala Ser Asn Leu Thr Gly Val
                245                 250                 255

Glu Ile Tyr Ser Leu Gln Thr Ser Arg Glu Pro Thr Pro Arg Gln Ser
            260                 265                 270

Ser Phe Asn Gln Ser Asp Phe Tyr Ser Met Phe Asn Gly Ser Lys Leu
        275                 280                 285

Ala Ser Pro Lys Gly Gln Pro Pro Val Ala Gly Gly Gly Gly Ala Arg
    290                 295                 300

Gly Gln Gly Leu Asp Glu Gln Val Ala Asn Lys Phe Lys Gly Gly Glu
305                 310                 315                 320

Ala Ala Ala Pro Tyr Pro Ala Pro Asn Pro Gly Met Met Met Pro Ala
                325                 330                 335

Pro Arg Lys Lys Glu Leu Gly Gly Ser Asn Ser Asn Ser Asp Lys Glu
            340                 345                 350

Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val Ser Glu Ala
        355                 360                 365

Asn Leu Arg Asn Ala Val Asn His Ala Ala Ser Thr Asp Phe Ala Ala
    370                 375                 380
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Ala Pro Pro Ala Ala Ala Thr Pro Arg Asp Gly Ala Thr Pro Arg Gly
385 390 395 400

Val Ser Gly Ser Val Thr Pro Val Met Lys Lys Asp Ala Ser Ser Gly
405 410 415

Ala Val Glu Val Glu Ile Glu Asp Gly Met Met Lys Ser Pro Ala Thr
420 425 430

Gly Leu Gly Ala Lys Phe Pro Val Ser Gly Ser Pro Tyr Val Ala Pro
435 440 445

Arg Lys Lys Gly Ala Asp Val Pro Gly Leu Glu Glu Ala Ala His Pro
450 455 460

Met Pro Pro Ala Ser Val Met Thr Arg Leu Ile Leu Ile Met Val Trp
465 470 475 480

Arg Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser Ser Leu Ile Gly Leu
485 490 495

Val Trp Ser Leu Val Ser Phe Arg Trp Asn Ile Gln Met Pro Thr Ile
500 505 510

Ile Lys Gly Ser Ile Ser Ile Leu Ser Asp Ala Gly Leu Gly Met Ala
515 520 525

Met Phe Ser Leu Gly Leu Phe Met Ala Leu Gln Pro Lys Ile Ile Ser
530 535 540

Cys Gly Lys Ser Val Ala Thr Phe Ala Met Ala Val Arg Phe Leu Thr
545 550 555 560

Gly Pro Ala Val Ile Ala Ala Thr Ser Ile Ala Val Gly Leu Arg Gly
565 570 575

Val Leu Leu His Val Ala Ile Val Gln Ala Ala Leu Pro Gln Gly Ile
580 585 590

Val Pro Phe Val Phe Ala Lys Glu Tyr Asn Cys His Pro Gln Ile Leu
595 600 605

Ser Thr Ala Val Ile Phe Gly Met Leu Val Ala Leu Pro Ile Thr Ile
610 615 620

Leu Tyr Tyr Val Leu Leu Gly Ile
625 630

<210> 39
<211> 447
<212> DNA
<213> Triticum aestivum

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cgatggtggc ggttcttcac ggcggagcag tgcggcgcca tcaacacgct ggtggtctgc 180
ttctccatgc ccttcttcac cttcgacttc gtggtccgcg ccgacccccta cgccatgaat 240
taccgcgtca tcgccgccga cgccgtcgcc aaacttctcg ccgtgctcgc cgcggccgtc 300
tgggcgcgct gcgccaaggc caaggccggc gcctactcgt ggtcatcacg gggttctccc 360
tgggcncgta caacaacacn ctcgtcgtcn gggtgccgct tctgggacgc caatttcngg 420
naattggggg gcanggactt tattttt 447

<210> 40
<211> 94
<212> PRT
<213> Triticum aestivum

<400> 40
Met Ile Ala Leu Gly Asp Ile Tyr Lys Val Val Glu Ala Met Ala Pro
1 5 10 15

Leu Tyr Phe Ala Leu Gly Leu Gly Tyr Gly Ser Val Arg Trp Trp Arg
20 25 30

Phe Phe Thr Ala Glu Gln Cys Gly Ala Ile Asn Thr Leu Val Val Cys
35 40 45

Phe Ser Met Pro Phe Phe Thr Phe Asp Phe Val Val Arg Ala Asp Pro
50 55 60

Tyr Ala Met Asn Tyr Arg Val Ile Ala Ala Asp Ala Val Ala Lys Leu
65 70 75 80

Leu Ala Val Leu Ala Ala Ala Val Trp Ala Arg Cys Ala Lys
85 90

<210> 41
<211> 415
<212> DNA
<213> Triticum aestivum

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tcgcccgcag agtgagccga ggccgagagc cggagcgcga gaggaagaag cagaggaggt 120
cgggcaagat gatcacgggc acggacttct accacgtgat gacggcggtg gtgccgctgt 180

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acgtggccat gatcctcgcc tacggctccg tcaagtggtg gggcatcttc acgccggacc 240
agtgctccgg gatcaaccgc ttcgtcgcgc tcttcgccgt gccgctcctc tccttccact 300
tcatctccac caacaacccc tacaccatga acctgcgctt catcgccgcc gacacgctgc 360
agaagctcat gatgctcgcc atgctcaacg cctggagcaa ctctcccgcc gcggc      415

&lt;210&gt;  42
&lt;211&gt;  91
&lt;212&gt;  PRT
&lt;213&gt;  Triticum aestivum

&lt;400&gt;  42
Met Ile Thr Gly Thr Asp Phe Tyr His Val Met Thr Ala Val Val Pro
 1               5                  10                  15

Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Lys Trp Trp Gly
            20                  25                  30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asn Pro
    50                  55                  60

Tyr Thr Met Asn Leu Arg Phe Ile Ala Ala Asp Thr Leu Gln Lys Leu
65                  70                  75                  80

Met Met Leu Ala Met Leu Asn Ala Trp Ser Asn
                85                  90

&lt;210&gt;  43
&lt;211&gt;  647
&lt;212&gt;  PRT
&lt;213&gt;  Arabidopsis thaliana

&lt;400&gt;  43
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 1               5                  10                  15

Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Arg Trp Trp Gly
            20                  25                  30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Val
        35                  40                  45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Ser Asn Asp Pro
    50                  55                  60

Tyr Ala Met Asn Tyr His Phe Leu Ala Ala Asp Ser Leu Gln Lys Val
65                  70                  75                  80

Val Ile Leu Ala Ala Leu Phe Leu Trp Gln Ala Phe Ser Arg Arg Gly
                85                  90                  95

Ser Leu Glu Trp Met Ile Thr Leu Phe Ser Leu Ser Thr Leu Pro Asn
            100                 105                 110

Thr Leu Val Met Gly Ile Pro Leu Leu Arg Ala Met Tyr Gly Asp Phe
        115                 120                 125
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Ser Gly Asn Leu Met Val Gln Ile Val Val Leu Gln Ser Ile Ile Trp
130 135 140

Tyr Thr Leu Met Leu Phe Leu Phe Glu Phe Arg Gly Ala Lys Leu Leu
145 150 155 160

Ile Ser Glu Gln Phe Pro Glu Thr Ala Gly Ser Ile Thr Ser Phe Arg
165 170 175

Val Asp Ser Asp Val Ile Ser Leu Asn Gly Arg Glu Pro Leu Gln Thr
180 185 190

Asp Ala Glu Ile Gly Asp Asp Gly Lys Leu His Val Val Val Arg Arg
195 200 205

Ser Ser Ala Ala Ser Ser Met Ile Ser Ser Phe Asn Lys Ser His Gly
210 215 220

Gly Gly Leu Asn Ser Ser Met Ile Thr Pro Arg Ala Ser Asn Leu Thr
225 230 235 240

Gly Val Glu Ile Tyr Ser Val Gln Ser Ser Arg Glu Pro Thr Pro Arg
245 250 255

Ala Ser Ser Phe Asn Gln Thr Asp Phe Tyr Ala Met Phe Asn Ala Ser
260 265 270

Lys Ala Pro Ser Pro Arg His Gly Tyr Thr Asn Ser Tyr Gly Gly Ala
275 280 285

Gly Ala Gly Pro Gly Gly Asp Val Tyr Ser Leu Gln Ser Ser Lys Gly
290 295 300

Val Thr Pro Arg Thr Ser Asn Phe Asp Glu Glu Val Met Lys Thr Ala
305 310 315 320

Lys Lys Ala Gly Arg Gly Gly Arg Ser Met Ser Gly Glu Leu Tyr Asn
325 330 335

Asn Asn Ser Val Pro Ser Tyr Pro Pro Pro Asn Pro Met Phe Thr Gly
340 345 350

Ser Thr Ser Gly Ala Ser Gly Val Lys Lys Lys Glu Ser Gly Gly Gly
355 360 365

Gly Ser Gly Gly Gly Val Gly Val Gly Gly Gln Asn Lys Glu Met Asn
370 375 380

Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val Ser Glu Ala Asn Ala
385 390 395 400

Lys Asn Ala Met Thr Arg Gly Ser Ser Thr Asp Val Ser Thr Asp Pro
405 410 415

Lys Val Ser Ile Pro Pro His Asp Asn Leu Ala Thr Lys Ala Met Gln
420 425 430

Asn Leu Ile Glu Asn Met Ser Pro Gly Arg Lys Gly His Val Glu Met
435 440 445

Asp Gln Asp Gly Asn Asn Gly Gly Lys Ser Pro Tyr Met Gly Lys Lys
450 455 460

Gly Ser Asp Val Glu Asp Gly Gly Pro Gly Pro Arg Lys Gln Gln Met
465 470 475 480

Pro Pro Ala Ser Val Met Thr Arg Leu Ile Leu Ile Met Val Trp Arg
485 490 495

Lys Leu Ile Arg Asn Pro Asn Thr Tyr Ser Ser Leu Phe Gly Leu Ala
500 505 510

Trp Ser Leu Val Ser Phe Lys Trp Asn Ile Lys Met Pro Thr Ile Met
515 520 525

Ser Gly Ser Ile Ser Ile Leu Ser Asp Ala Gly Leu Gly Met Ala Met
530 535 540

Phe Ser Leu Gly Leu Phe Met Ala Leu Gln Pro Lys Ile Ile Ala Cys
545 550 555 560

Gly Lys Ser Val Ala Gly Phe Ala Met Ala Val Arg Phe Leu Thr Gly
565 570 575

Pro Ala Val Ile Ala Ala Thr Ser Ile Ala Ile Gly Ile Arg Gly Asp
580 585 590

Leu Leu His Ile Ala Ile Val Gln Ala Ala Leu Pro Gln Gly Ile Val
595 600 605

Pro Phe Val Phe Ala Lys Glu Tyr Asn Val His Pro Asp Ile Leu Ser
610 615 620

Thr Ala Val Ile Phe Gly Met Leu Val Ala Leu Pro Val Thr Val Leu
625 630 635 640

Tyr Tyr Val Leu Leu Gly Leu
645

<210> 44
<211> 622
<212> PRT
<213> Arabidopsis thaliana

<400> 44
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Leu Tyr Val Ala Met Ile Leu Ala Tyr Gly Ser Val Lys Trp Trp Lys
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Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ala Ala Asn Asn Pro
50 55 60

Tyr Ala Met Asn Leu Arg Phe Leu Ala Ala Asp Ser Leu Gln Lys Val
65 70 75 80

Ile	Val	Leu	Ser	Leu	Leu	Phe	Leu	Trp	Cys	Lys	Leu	Ser	Arg	Asn	Gly
				85					90					95	
Ser	Leu	Asp	Trp	Thr	Ile	Thr	Leu	Phe	Ser	Leu	Ser	Thr	Leu	Pro	Asn
			100					105					110		
Thr	Leu	Val	Met	Gly	Ile	Pro	Leu	Leu	Lys	Gly	Met	Tyr	Gly	Asn	Phe
		115					120					125			
Ser	Gly	Asp	Leu	Met	Val	Gln	Ile	Val	Val	Leu	Gln	Cys	Ile	Ile	Trp
	130					135					140				
Tyr	Ile	Leu	Met	Leu	Phe	Leu	Phe	Glu	Tyr	Arg	Gly	Ala	Lys	Leu	Leu
145					150					155					160
Ile	Ser	Glu	Gln	Phe	Pro	Asp	Thr	Ala	Gly	Ser	Ile	Val	Ser	Ile	His
				165					170					175	
Val	Asp	Ser	Asp	Ile	Met	Ser	Leu	Asp	Gly	Arg	Gln	Pro	Leu	Glu	Thr
			180					185					190		
Glu	Ala	Glu	Ile	Lys	Glu	Asp	Gly	Lys	Leu	His	Val	Thr	Val	Arg	Arg
		195					200					205			
Ser	Asn	Ala	Ser	Arg	Ser	Asp	Ile	Tyr	Ser	Arg	Arg	Ser	Gln	Gly	Leu
	210					215					220				
Ser	Ala	Thr	Pro	Arg	Pro	Ser	Asn	Leu	Thr	Asn	Ala	Glu	Ile	Tyr	Ser
225					230					235					240
Leu	Gln	Ser	Ser	Arg	Asn	Pro	Thr	Pro	Arg	Gly	Ser	Ser	Phe	Asn	His
				245					250					255	
Thr	Asp	Phe	Tyr	Ser	Met	Met	Ala	Ser	Gly	Gly	Gly	Arg	Asn	Ser	Asn
			260					265					270		
Phe	Gly	Pro	Gly	Glu	Ala	Val	Phe	Gly	Ser	Lys	Gly	Pro	Thr	Pro	Arg
		275					280					285			
Pro	Ser	Asn	Tyr	Glu	Glu	Asp	Gly	Gly	Pro	Ala	Lys	Pro	Thr	Ala	Ala
	290					295					300				
Gly	Thr	Ala	Ala	Gly	Ala	Gly	Arg	Phe	His	Tyr	Gln	Ser	Gly	Gly	Ser
305					310					315					320
Gly	Gly	Gly	Gly	Gly	Ala	His	Tyr	Pro	Ala	Pro	Asn	Pro	Gly	Met	Phe
				325					330					335	
Ser	Pro	Asn	Thr	Gly	Gly	Gly	Gly	Gly	Thr	Ala	Ala	Lys	Gly	Asn	Ala
			340					345					350		
Pro	Val	Val	Gly	Gly	Lys	Arg	Gln	Asp	Gly	Asn	Gly	Arg	Asp	Leu	His
		355					360					365			
Met	Phe	Val	Trp	Ser	Ser	Ser	Ala	Ser	Pro	Val	Ser	Asp	Val	Phe	Gly
	370					375					380				
Gly	Gly	Gly	Gly	Asn	His	His	Ala	Asp	Tyr	Ser	Thr	Ala	Thr	Asn	Asp
385					390					395					400

His Gln Lys Asp Val Lys Ile Ser Val Pro Gln Gly Asn Ser Asn Asp
405 410 415

Asn Gln Tyr Val Glu Arg Glu Glu Phe Ser Phe Gly Asn Lys Asp Asp
420 425 430

Asp Ser Lys Val Leu Ala Thr Asp Gly Gly Asn Asn Ile Ser Asn Lys
435 440 445

Thr Thr Gln Ala Lys Val Met Pro Pro Thr Ser Val Met Thr Arg Leu
450 455 460

Ile Leu Ile Met Val Trp Arg Lys Leu Ile Arg Asn Pro Asn Ser Tyr
465 470 475 480

Ser Ser Leu Phe Gly Ile Thr Trp Ser Leu Ile Ser Phe Lys Trp Asn
485 490 495

Ile Glu Met Pro Ala Leu Ile Ala Lys Ser Ile Ser Ile Leu Ser Asp
500 505 510

Ala Gly Leu Gly Met Ala Met Phe Ser Leu Gly Leu Phe Met Ala Leu
515 520 525

Asn Pro Arg Ile Ile Ala Cys Gly Asn Arg Arg Ala Ala Phe Ala Ala
530 535 540

Ala Met Arg Phe Val Val Gly Pro Ala Val Met Leu Val Ala Ser Tyr
545 550 555 560

Ala Val Gly Leu Arg Gly Val Leu Leu His Val Ala Ile Ile Gln Ala
565 570 575

Ala Leu Pro Gln Gly Ile Val Pro Phe Val Phe Ala Lys Glu Tyr Asn
580 585 590

Val His Pro Asp Ile Leu Ser Thr Ala Val Ile Phe Gly Met Leu Ile
595 600 605

Ala Leu Pro Ile Thr Leu Leu Tyr Tyr Ile Leu Leu Gly Leu
610 615 620

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<213> Triticum aestivum

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aggaggtcgg gcaagatgat cacgggcacg gacttctacc acgtgatgac ggcggtggtg 180
ccgctgtacg tggccatgat cctcgcctac ggctccgtca agtggtgggg catcttcacg 240
ccggaccagt gctccgggat caaccgcttc gtcgcgctct tcgccgtgcc gctcctctcc 300
ttccacttca tctccaccaa caacccctac accatgaacc tgcgcttcat cgccgccgac 360
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ggcag 425

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20 25 30

Ile Phe Thr Pro Asp Gln Cys Ser Gly Ile Asn Arg Phe Val Ala Leu
35 40 45

Phe Ala Val Pro Leu Leu Ser Phe His Phe Ile Ser Thr Asn Asn Pro
50 55 60

Tyr Thr Met Asn Leu Arg Phe Ile Ala Ala Asp Thr Leu Gln Lys Leu
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Met Met Leu Ala Met Leu Thr Ala Trp Ser His Leu Ser Arg Arg Gly
85 90 95

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<212> DNA
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atgggcatcc cgctgctcaa gggcatgtac ggcgacttct ccggcagcct catggtgcag 180
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gacatctact cccggcggtc catggggttc tccagcacca cgccgcggcc cagcaacctg 480
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gcgttcggcc tgcgcacggg cgccacgccc aggccgtcca actacgagga ggacccgcag 660
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gccaagggcc tcaagaaggc ggccaatggg caggccaagg gcgaggacgg caaggaccta 780
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<210> 48
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Leu Ser Arg Arg Gly Cys Leu Glu Trp Thr Ile Thr Leu Phe Ser Leu
20 25 30

Ser Thr Leu Pro Asn Thr Leu Val Met Gly Ile Pro Leu Leu Lys Gly
35 40 45

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Met Tyr Gly Asp Phe Ser Gly Ser Leu Met Val Gln Ile Val Val Leu
     50                  55                  60

Gln Cys Ile Ile Trp Tyr Thr Leu Met Leu Phe Met Phe Glu Tyr Arg
65                  70                  75                  80

Gly Ala Arg Ile Leu Ile Thr Glu Gln Phe Pro Asp Thr Ala Gly Ala
                85                  90                  95

Ile Ala Ser Ile Val Val Asp Pro Asp Val Val Ser Leu Asp Gly Arg
            100                 105                 110

Asn Asp Ala Ile Glu Thr Glu Ala Glu Val Lys Glu Asp Gly Lys Ile
        115                 120                 125

His Val Thr Val Arg Arg Ser Asn Ala Ser Arg Ser Asp Ile Tyr Ser
    130                 135                 140

Arg Arg Ser Met Gly Phe Ser Ser Thr Thr Pro Arg Pro Ser Asn Leu
145                 150                 155                 160

Thr Asn Ala Glu Ile Tyr Ser Leu Gln Ser Ser Arg Asn Pro Thr Pro
                165                 170                 175

Arg Gly Ser Ser Phe Asn His Thr Asp Phe Tyr Ser Met Val Gly Arg
            180                 185                 190

Ser Ser Asn Phe Ala Ala Gly Asp Ala Phe Gly Leu Arg Thr Gly Ala
        195                 200                 205

Thr Pro Arg Pro Ser Asn Tyr Glu Glu Asp Pro Gln Gly Lys Ala Asn
    210                 215                 220

Lys Tyr Gly Gln Tyr Pro Ala Pro Asn Pro Ala Met Ala Ala Gln Pro
225                 230                 235                 240

Ala Lys Gly Leu Lys Lys Ala Ala Asn Gly Gln Ala Lys Gly Glu Asp
                245                 250                 255

Gly Lys Asp Leu His Met Phe Val Trp Ser Ser Ser Ala Ser Pro Val
            260                 265                 270

Ser Asp Val Phe Gly Asn Gly Ala Ala Glu Tyr Asn Asp
        275                 280                 285
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